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Advances and Challenges in Insect Rearing

Edited by E. G. King and N. C. Leppla

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Abstract

This book contains greatly elaborated and revised versions of 36 papers presented at a conference sponsored by the U.S. Department of Agriculture and the Insect Rearing Group in March 1980. These papers deal comprehensively with the genetics of reared insects, especially the decline in variability and performance and ways to guard against both; diets and containers; engineering problems and solutions for insect-rearing facilities and systems; the discovery and control of pathogens and micro-organisms in insect rearing; actual rearing systems for a diverse selection of insects intended for a variety of uses; and the management of insect-rearing programs, including data-processing techniques, systems management, and quality control.

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Preface

✓ This book is an elaboration of papers and discussions from the conference "Advances and Challenges in Insect Rearing" held in Atlanta, Ga., on March 4-6, 1980. We have retained the original organization of the conference in the book's six sections. The first four sections are basic to insect rearing, and the fifth conveys the state of the art with descriptions of several rearing systems. The sixth and final section discusses various topics important to management of insect-rearing systems. In the "Message From the Conference Chairman," R. F. Moore outlines the questions addressed by the conference and discusses its origins and the reasons for its organization. And the rationale and need for insect rearing and research is elegantly presented by E. F. Knipling in the Foreword, based on his keynote address. Finally, many recommendations about further research and program needs developed from the conference, and these are included in "Recommendations of the Conference."

Thanks are due to many people for support of the conference and for completion of this book. Certainly, R. F. Moore, the conference chairman; section leaders; and authors of the various papers deserve tribute for their contributions. We also appreciate the support of administrators in the U.S. Department of Agriculture, State agricultural experiment stations, and the private sector.

E. G. King
and
N. C. Leppla,
Research entomologists,
Agricultural Research Service

Message From the Conference Chairman

The impetus for this conference was an item by Tom O'Dell in the March 8, 1978, issue of *Frass*, the Insect Rearing Group's newsletter. He indicated that many people were interested in a workshop on rearing insects. The last conference on insect nutrition and rearing was sponsored by the U.S. Department of Agriculture (USDA) in March 1963; since then, many advances and changes have occurred. Because I am a technical adviser for insect rearing and nutrition on the national research program "Non-Commodity Research for Insect Control" of USDA's Agricultural Research Service, it was appropriate for me to support a workshop on this topic. I contacted Tom and others to determine if plans were being made to hold such a workshop. On learning that no active plans were in progress, I invited 10 interested scientists to a meeting in Washington, D.C., on April 27, 1978, to determine if a workshop should be developed.

Their interest, enthusiasm, and concern left no doubt that there should be a workshop; but limiting the subject and developing a format proved difficult. What were to be the purpose, subject matter, and scope, and how was the conference to be documented? There were concerns that insect rearing was not recognized as an important area of entomology, that administrative support for rearing was inadequate, and that much information was not readily available. How could all these interests be satisfied in one short conference?

After some prolonged discussions, we arrived at the following objectives:

1. To assemble the scientific principles of insect rearing that have been established in recent years. These would include guidelines for establishing and maintaining colonies of insects for specific purposes.
2. To identify problem areas in insect-rearing programs and develop specific recommendations for problem solving. These recommendations would include procedures for research, development, and implementation.
3. To establish the complexity and integrity of insect rearing as a field of scientific research.
4. To document the state of the art of insect rearing and establish a reference for direction of the science through publication of the conference proceedings.

Rearing of insects has been regarded as nontechnical labor, so criticism of the research effort expended on it has been considerable. This attitude has persisted be-

cause early programs reared insects that were used primarily for screening pesticides and for associated research. At that time, we depended too much on chemicals for controlling insects; now, with the advent of alternate methods of control, insect rearing has assumed new and major importance. Since most of these methods require the ability to rear insects of specified quality, the following highly technical questions have emerged:

1. How are laboratory-reared insects different from wild ones?
2. What is the most economical means of meeting the nutritional requirements of insects?
3. What are the most labor-efficient methods of rearing insects?
4. How can we be certain these insects will perform adequately in a control program?

These questions have been addressed in the six complementary sections of the program:

Section 1, "Establishment and Maintenance of Insect Colonies Through Genetic Control," discusses some genetic reasons for the differences observed between laboratory and wild insects. The organizing committee expects that some principles and guidelines for colonizing insects will be found in this section. There will also be unanswered questions that will require future research. For example, can we, now or in the future, have breeding programs to develop strains of insects for special purposes?

Section 2, "Diets and Containerization for Insect Rearing," gives information on the ingredients of diets and on methods of handling and using artificial diets. Quality and cost of dietary ingredients increase in importance as more insects are reared. Diets that are more balanced in protein, carbohydrates, lipids, vitamins, and minerals are needed for more efficient utilization; an economical substitute for agar is also vital.

Section 3, "Engineering for Insect Rearing," recognizes the contribution of engineers to reducing the labor required to handle insects, a major expense in rearing. In the recent past, facilities for rearing insects were modified rooms or buildings; today, however, insectaries are sometimes specially designed. Special designs are necessary because the needs of the rearing programs vary. Some facilities produce large quantities of insects for control purposes. Others produce small research cultures. And still others produce intermediate amounts.

Mass-rearing programs will probably always need specially designed facilities. But, perhaps we are approaching the time when a standardized facility can be designed for intermediate research and small-scale production.

Section 4, "Control of Pathogens and Microbial Contaminants in Insect Rearing," demonstrates the importance of controlling insect pathogens. There is increasing evidence that microbial contaminants affect both the quality of laboratory-reared insects used for alternate methods of control and the results of research obtained with these insects.

Section 5, "Production, Use, and Quality Testing in Insect Rearing," details the rearing of several insect species. Even though the insects discussed in this section are mainly phytophagous, the general rearing principles also apply to entomophages. Quality testing, for example, is of great importance for any insects used in alternate methods of control. Quality tests in the screwworm, *Cochliomyia hominivorax* (Coquerel), program measured the size of the last-instar larvae, survival in the laboratory, and mating competitiveness. Measurements such as rate of development to pupal or adult stages, number of eggs produced, and yield of adults are used in other programs. These are rather gross indicators; and, for some species, several diets or rearing conditions will produce apparently equivalent insects. Presently, we are looking at more subtle tests such as electrophysiological response to light and sound, pheromone production, locomotor activity, enzyme levels, and other variables that seem likely

to affect performance. These tests should tell us how the insects vary in these qualities from week to week. But, to date, we have few direct relationships between laboratory tests and field performance. A further limitation to current quality testing is that the test results are obtained after the insects have been released. A goal of quality testing should be to develop tests that will predict performance.

Section 6, "Management of Insect-Rearing Systems," brings together all the complexities of the processes of insect rearing and use. This systematization is especially apparent in the large programs designed to support alternate methods of control. These massive enterprises, requiring procedures based on current research, must be managed by qualified professionals.

Finally, I want to acknowledge the support and assistance of the members of the Organizing and Planning Committee, their research leaders, area directors, the National Program Staff, and regional administrative officers. I especially want to thank the section leaders and the others who assisted unofficially for their interest and dedication. Their helpful assistance and encouragement has made development of this conference a real pleasure.

R. F. Moore,
Supervisory research entomologist,
Agricultural Research Service

Foreword

What Colonization of Insects Means to Research and Pest Management

Since the ability to rear insects is important to virtually every aspect of entomological research, and I am interested in all aspects of entomological research, it is a pleasure to attend this conference to keep abreast of the field's problems and progress. Insect-rearing technology can make direct contributions to the management of many destructive pests by enabling strategies that are sound in principle, economically advantageous, and virtually without hazard to nontarget organisms. But, some scientists have been critical of the amount of research effort devoted to insect rearing. So, in my address at the 1977 annual meeting of the Entomological Society of America, I cited advances in insect rearing as being among the most important developments in entomology during the last few decades. I will reiterate this viewpoint here because this field continues to warrant high priority.

Most entomologists take for granted our present capability for maintaining colonies of insects. But, when my career began in the early 1930's, colonies were limited to a few easy-to-rear species such as fruit flies; house flies, *Musca domestica* Linnaeus; cockroaches; and certain stored-product insects. Later, in 1936, the screwworm, *Cochliomyia hominivorax* (Coquerel), was reared on an artificial diet developed by R. Melvin and R. C. Bushland of the U.S. Department of Agriculture. This milestone made possible the rapid screening of hundreds of candidate chemicals and other formulations for effectively treating livestock wounds. Eventually it facilitated suppression of the screwworm by genetic means.

An early experience that also impressed me with the vital role of insect rearing in advancing applied entomology occurred during World War II. The laboratory where I worked at Orlando, Fla., was engaged in an urgent program to develop insecticides and repellents for protecting military personnel from pests and disease vectors in various parts of the world. But methods had not been devised for rearing the body louse, *Pediculus humanus humanus* Linnaeus, and malaria-transmitting *Anopheles* mosquitoes on a useful scale. Other pests (such as bed bugs, *Cimex lectularius* Linnaeus; fleas; ticks; and cockroaches) could be reared but not in appreciable numbers. Therefore, with techniques perfected by G. H. Culpepper, thousands of body lice were maintained by permitting them to feed on human hosts twice each day, and large colonies of the other pests were developed.

These colonies made it possible to screen thousands of candidate insecticides and repellents in a short time. The most promising materials were intensively investigated, and this work led to new control measures for a wide range of vectors and pests affecting man in many parts of the world.

After World War II, scientists with the U.S. Department of Agriculture were encouraged to undertake research on methods for rearing various crop pests. At that time, many laboratories were poorly designed and equipped for this purpose. So improvement of facilities was given high priority. Rapid progress was made; much of it could be attributed to basic studies on insect nutrition conducted by E. S. Vanderzant in cooperation with scientists at Texas A&M University. These studies led to laboratory colonization of the boll weevil, *Anthonomus grandis grandis* Boheman; pink bollworm, *Pectinophora gossypiella* (Saunders); and *Heliothis* spp. Today, through the combined efforts of many entomologists and engineers with Federal and State institutions and private industry, it is possible to produce these major pests by the hundreds of millions. Early advances in research on insect colonization are described in the book "Insect Colonization and Mass Production," edited by C. N. Smith (Academic Press, New York, 1966).

It is difficult to evaluate the benefits of insect rearing to the advancement of entomological research. House fly colonization alone has facilitated discovery of many new insecticides and refinement of their formulations. Until appropriate insect colonies became available, important studies on insect physiology could not be undertaken, research on the genetics of economic pests was handicapped, and identification and synthesis of sex pheromones were delayed. The ability to rear insects facilitated research on plant resistance and made possible various field studies on the dispersal and behavior of insects. Finally, the field of biological control has been greatly advanced by having methods of rearing parasites and predators for introduction or augmentation and by the propagation of hosts for insect pathogens.

While development of rearing methods has been vital to the advancement of all aspects of entomology, basic and applied, the use of this technology to produce insects for destruction of their own kind offers an effective alter-

native for controlling certain major pests. But appraisal of this approach requires both accurate information on the number of pest insects and the ability to inexpensively mass-produce enough good quality insects to adequately flood the target population. Since information on absolute numbers of insects per unit of area is lacking for most major pests, indirect estimates must be used. Once computed, such estimates must be related to the cost of rearing and releasing the quantities necessary to reach the required degree of suppression. In most cases, the pests are so abundant at normal density ranges that managing them by releasing relatively few sterile or genetically altered conspecifics is impractical. Therefore, other means must first be used to suppress these populations. Despite this requirement, I think the approach offers great promise, particularly considering current losses and the need for suppressive measures that avoid or minimize the ecological disruptions and costs associated with the use of broad-spectrum chemicals.

The advent of this two-step process, use of one technique to reduce high population densities followed by another that is more efficient at low levels, led to a critical analysis of the mechanisms of various techniques of insect control and of how compatible methods might be appropriately integrated for insect suppression. I have detailed this fundamental approach in my book, "The Basic Principles of Insect Population Suppression and Management" (U.S. Department of Agriculture, Agriculture Handbook 512, 1979). I am confident that the use of two or more methods of suppression, together or in sequence, will eventually lead to more effective and more acceptable insect-management systems. The use of biological organisms may be one of the most practical and desirable techniques to employ in such management procedures. If so, the development of mass-rearing technology will become increasingly more important.

When the potential of genetic engineering is fully appreciated, there should be greater interest in the practical use of genetic control for insect suppression. In fact, simulation models I developed in cooperation with W. Klasen show that several techniques of genetic manipulation offer prospects of greater efficiency than the conventional sterile-insect technique. This appraisal is based on results of genetic effects reported by several investigators. For example, M. L. Laster of the Mississippi State Agricultural Experiment Station found that the tobacco budworm, *H. virescens* (Fabricius), crossed with a closely related species, *H. subflexa* Guenée, produces sterile male progeny. Females, however, are fertile and can be backcrossed with tobacco budworm males for an apparently infinite number of generations to produce more sterile males and fertile females. This unique type of genetic action is now under investigation at the U.S.

Agricultural Research Service's Southern Field Crop Insect Management Laboratory at Stoneville, Miss. Likewise, many investigators, working on several moth species, have shown that parent moths receiving substerilizing dosages of irradiation produce progeny that have higher levels of sterility than their parents and that some adverse effects may persist for several generations. Other promising methods of genetic engineering include production of nondiapausing strains and conditional lethal factors. Regardless of the genetic mechanism that may be involved, however, the full potential of genetic suppressive measures will not be realized until insects that are reasonably competitive with their natural counterparts can be mass-produced.

Even though interest in mass rearing has centered on developing genetic-control methods, during the past 15 years entomologists have become increasingly interested in more extensive and dependable use of biological-control agents. To appraise both the merits and the limitations of natural biological-control organisms, I have made extensive use of models that simulate codeveloping parasite-host populations. While the accuracy of such appraisals can be limited, this effort yielded the following conclusions: Self-perpetuating populations of many parasites cannot consistently suppress their hosts below damaging levels because of the actions and interactions of several natural regulating forces; dependability of these parasites can be greatly increased, however, by augmenting their numbers continuously or at strategic times in the host cycle; and, based on estimates of the host-finding efficiency of certain parasites and on projected costs for their mass production, using augmentation offers outstanding potential for effectively managing a wide range of major pests. Equally important, augmentation of natural predators and parasites should have little or no effect on the environment.

I believe the prospects are excellent that populations of many of the world's major agricultural insect pests and disease vectors can be controlled on a regional or ecosystem basis at levels below those that are economically damaging; this control can be achieved if various suppression techniques that are already available or that can be developed are properly integrated. In recent years, important advances have been made on various methods of suppression; these advances include more selective chemicals, resistant crop varieties, and various types of attractants that may be useful for suppression and may also provide highly sensitive methods for population detection and assessment. Biological organisms, including genetically altered insects, insect parasites, insect predators and microbial agents, may be among the most practical and desirable components to use in systems for area-wide population management. But the usefulness of biological

organisms for such purposes will depend not only on efficient mass-production capabilities but also on the quality of the organisms produced.

There is also an urgent need for research on the ecological matters that are highly relevant to the concept of regional management. Many entomologists have given little consideration to this regional approach because the research and operational problems involved in developing and executing such a pest-management procedure are so large. More definitive information is needed on numbers of pest insects in natural or reduced populations, the rate and extent of dispersal, mating behavior, and the dynamics of various pest populations. But getting enough money for this necessary research is difficult. Yet I feel that the alternatives should be critically analyzed. Despite the availability of a wide variety of highly effective insecticides, we know through several decades of experience that a limited and uncoordinated attack on segments of pest populations, farm to farm and crop to crop, has had no major impact on the abundance of major annually recurring pests. They are as numerous and pose the same threat to production today as they did several decades ago, especially such important pests as the boll weevil; *Heliothis* complex; pink bollworm; codling moth, *Laspeyresia pomonella* (Linnaeus); fall armyworm, *Spodoptera frugiperda* (J. E. Smith); cabbage looper, *Trichoplusia ni* (Hübner); tropical fruit flies (Tephritidae); sugarcane borer, *Diatraea saccharalis* (Fabricius); European corn borer, *Ostrinia nubilalis* (Hübner); and various

flies affecting livestock. And we still depend mostly on ecologically disruptive insecticides for controlling these pests when they reach damaging numbers. So I share the opinion of more and more entomologists that there is every justification for full exploration of the concept of regional management. Methods of colonizing and mass producing biological organisms of various kinds are likely to be especially important in achieving this goal.

The success in suppressing a wide-ranging pest like the screwworm, on a regional basis and with great economic benefits, should not be regarded as exceptional. I feel that the same opportunities exist for managing many other major pests with suppression methods that would eliminate crop losses and cost less than insecticides. And techniques likely to be most useful in systems for managing pest populations are generally pest specific and should do little or no damage to the environment. Therefore, conferences such as this are important and necessary so that investigators engaged in research on insect colonization and mass production can report on progress and problems that may be applicable to a wide range of species.

E. F. Knipling,
Expert consultant in pest management,
Agricultural Research Service

Recommendations of the Conference

Discussion during the conference revealed that much critical research still must be done to achieve the ability to mass-rear high-quality insects consistently. Section leaders consulted with their colleagues during and after the conference, and the following recommendations were formulated. These recommendations can provide direction to scientists and administrators for establishing focus and priorities for future research on insect rearing.

Establishment and Maintenance of Insect Colonies Through Genetic Control

The maintenance of insect colonies has become necessary and vital to modern pest-management strategies, including sterile-insect release, genetic control (such as hybrid sterility, conditional lethal mutations, and translocation sterility), and biological control (such as parasites, predators, viruses, and bacteria). The establishment of colonies and adaptation of insects to laboratory conditions result in genetic changes with unknown effects on the performance of the insect. Some effects of domestication are probably unavoidable, but certain precautions can minimize their impact. The following recommendations are made to emphasize steps that could be taken to avoid serious genetic alteration of colonized species and to suggest areas of research that should be supported.

1. How genetic selection affects performance of released insects depends on their intended function. Neither sterile-male releases that require insemination of wild mates nor augmentative releases of natural enemies to control extant populations would need the same genetic diversity as a release to produce an in-field reproductive population or as use of a colonized insect for viral or bacterial production.

Recommendations: Support research to determine the requirements for genetic diversity in laboratory-reared insects used for various purposes. Determine the environmental conditions that will select for the best genotypic constitution. Then, develop and perfect methods for maintaining these conditions, including ways to alter temperature and light cycles, maintain humidity, control effects of nutrition on selection and performance, and establish mating conditions that are as natural as possible.

2. Laboratory domestication, the restriction of population size, and use of artificial diets inevitably lead to differences in gene frequency (caused by genetic drift and selection) between the native and colonized populations.

Therefore, there should be mechanisms to monitor such changes, evaluate their effects, and compensate for any that are harmful. Such criteria as isozyme variation, morphological mutations, chromosomal variation, behavioral traits (including pheromone response, physical activity, and reproductive processes), visual acuity, and physical characteristics (such as body weight, wing length, bristle number, temperature tolerance, and diapause response) show genetic variation in insect populations.

Recommendations: Each program involving the use of colonized insects should employ a scientist trained in genetic techniques (chromosomal analysis, electrophoresis, selection, genetic analysis, etc.) so that as many genetic variables as possible may be measured in the base population and during generations of domestication. Parallel behavioral characteristics need to be studied so that the relationship between the genetic traits and performance of the released insect can be established. As observations on changes in genetic variation and behavior accumulate, correlations can be made with changes in performance of domesticated populations. This information, in turn, will increase our ability to control such changes and predict insect performance.

3. Efficiency of laboratory-reared insects in applications for population control is affected by at least three significant factors: genetic selection during domestication, genetic changes in the native population resulting from control procedures, and environmental and geographic variation in the native population. Each of these problems must be studied from the viewpoint of population genetics.

Recommendations: Give high priority to expansion of expertise in insect population genetics (either by employing trained scientists or retraining existing personnel). Support basic field studies on pest species relating to their geographic variation and reproduction mechanisms, resistance, and preferred host and alternate host selection. Initiate and expand areawide programs to measure the population dynamics of target species.

Diets and Containerization for Insect Rearing

1. Much progress has been made in formulating artificial diets for rearing insects; but optimal diets need to be developed for production of high-quality insects. An insect's adaptability permits it to establish and reproduce

under laboratory conditions, but some genetic traits are lost. Performance of these insects is also affected by the interaction between genetic traits and the effects of nutrition and dietetics. The precise influence of each of these on the insect is seldom determined. Also, many species must still be reared on their host plants, and parasites and predators usually require their host insect. Quality and cost of nutrients are increasing in importance. And we need to standardize, as much as possible, storage of ingredients, ways to insure adequate nutritive composition, diet preparation, and conduct of associated rearing methods.

Recommendations: Improve and refine existing artificial diets to meet optimal performance criteria for insects reared in the laboratory. Develop artificial diets for rearing representative species of beneficial and pest insects. Support research to reduce the cost of artificial diets, particularly for mass rearing. Alternative, low-cost primary nutrient sources, especially gelling and bulking agents, need to be evaluated and used. Standardize diets and rearing procedures for representative insects. Develop guidelines to insure uniform quality of dietary ingredients, diet preparation, rearing procedures, and environmental conditions.

2. Primary nutrients such as nonrefined protein (wheat germ or cottonseed meal) and refined ingredients (casein, vitamins, or agar) vary in quality because of contamination with micro-organisms, improper storage, poor containers, different suppliers, or other unknown factors. Existing techniques for monitoring quality, measuring microbial levels, etc., should be used consistently in rearing operations.

Recommendations: Develop specifications that define the quality of diet ingredients. Establish procedures to monitor or assay the quality of commonly used dietary ingredients from various sources and determine if there are significant deviations from specified tolerances. Define and conduct research to determine the optimal storage conditions and shelf life for diet ingredients.

3. In each program for rearing insects, scientists develop their own procedures for measuring and mixing diet ingredients, preparing (heating) and dispensing diet, and operating equipment. Studies to establish optimal conditions and equipment for a broad range of insects have been inadequate.

Recommendation: Support research to determine optimal methods and equipment for diet preparation to avoid loss of nutrients and retain the desired physical properties.

4. Insect-rearing containers are generally adapted from commercially available cups or boxes designed for other purposes. These are not always best suited to the insect, are especially vulnerable to increased cost, and may be discontinued or changed at any time. So an entire rearing system may depend on suboptimal containers.

Recommendations: Develop specifications for improved insect-rearing containers. Evaluate the relative merits of disposable and reusable containers. Determine the costs and benefits of custom-made containers.

Engineering for Insect Rearing

1. Efficient production and use of insects require engineering expertise. Joint research of an engineer and entomologist results in, for example, more efficient rearing facilities, better environmental conditions for insects and personnel, automation of rearing procedures and diet handling (with major savings in cost of labor), and better working conditions through control of insect and dietary waste products. Those engineers with experience in these areas have not been used effectively. And there have been few efforts to provide them with training by exposure to existing insect facilities and programs.

Recommendations: Increase engineering support of insect rearing by assigning additional engineers to these programs. Provide engineers with training in existing facilities so they will be familiar with the many problems of rearing insects.

2. Facilities are often designed with limited knowledge of the problems of insect rearing and of solutions that have been devised by other engineers and entomologists.

Recommendation: Provide for consultation with engineers and entomologists when designing or remodeling insectaries.

3. Research in insect rearing has recognized and solved many problems. Some of these are common to all insects, some to certain orders, and others to certain species. Efficient use and extension of this information to all insect rearing is needed.

Recommendations: Develop engineering standards for the problems that are common to many insects and for which solutions have been devised, such as clean-air systems, environmental controls, backup systems, traffic-flow patterns, and diet-handling systems. Provide a standard terminology that will be understood by both engineers and entomologists.

4. When rearing insects, one often encounters unusual

and unexpected environmental problems. For example, maintenance of a proper level of humidity to prevent microbial contamination in the diet and provision for a temperature gradient from floor to ceiling may interfere with uniformity of insect development.

Recommendation: Support research on the best environmental conditions for rearing insects and on methods to maintain these conditions in the facility.

Control of Pathogens and Microbial Contaminants in Insect Rearing

1. Widespread use of laboratory-reared insects in alternate methods of control and in support of basic and applied research requires that they be of a consistently good quality. Gross and lethal microbial contamination of the insects and their diets have been recognized since the beginning of insect rearing, and modern antimicrobial agents now eliminate many of these problems. But sublethal microbial infections are often not detected, and there is evidence that these diseases may subtly affect development, behavior, and reproduction. The antimicrobial inhibitors may also affect insect performance.

Recommendations: Support research to devise methods for detecting sublethal micro-organisms and for determining how they affect the behavior of laboratory-reared insects. Also, support research to recognize how antimicrobial agents affect insects and to discover new and more effective microbial inhibitors. Assemble management guidelines for preventing and controlling contamination.

2. Information on diseases of laboratory-reared insects is not widely circulated. Frequently, unknown diseases may destroy a colony. Compilation of data on the sources, prevention, and elimination of insect diseases is required.

Recommendations: Conduct periodic workshops to familiarize those engaged in rearing insects with information on disease recognition, diagnostic techniques, and therapeutic methods. Publish a manual that would be a source of this information. Support research to catalog and identify micro-organisms that infect laboratory-reared insects. Discover effective methods for prevention and control of diseases that affect insects. These methods might include improvements in the design of insect-rearing facilities and control of micro-organisms in components of the diet, or in the prepared diet, or in the insect and its byproducts.

3. A colony of insects may be free of disease for a long time and then suddenly become susceptible to and be

debilitated by organisms that were previously non-pathogenic.

Recommendation: Determine factors in the environment, diet, and breeding of insects that promote biological vigor and reduce the incidence of diseases.

Production, Use, and Quality Testing in Insect Rearing

1. Use of laboratory-reared insects for basic research and evaluation of current and alternate methods of control have created demand for a central source of expertise in all phases of rearing. Expertise in engineering, pathology, physiology, genetics, insect nutrition, etc., are required to supply insects, solve rearing problems, and expedite development of new facilities and programs.

Recommendation: Determine the feasibility of establishing a central insect-rearing research center.

2. Laboratory insects differ from their wild parent population in behavioral, biological, and physiological characteristics. But there are few tests of quality that compare these characteristics in laboratory and wild populations and even fewer that relate differences to the performance or usefulness of the laboratory insects. These data are essential for precise interpretation of results of studies obtained with laboratory-reared insects.

Recommendations: Initiate an intensive research program to discover, evaluate, and define the biological, behavioral, and physiological characteristics that describe insect quality, especially those characteristics of major insect pests. The scope of the program should include: The identification of characteristics to be measured; the development of laboratory techniques to assess insect quality, which can be directly correlated with performance under natural conditions; and what threshold levels (physiological, behavioral, ecological, physical, and genetic) to accept and when and how to accept them.

3. Considering the worldwide multiplicity of uses for various laboratory cultures, standardized tests must be developed and studies must be conducted to characterize established insect colonies reared with standard techniques. The availability of performance standards for laboratory populations would promote communication and cooperation between scientists working with the same species and would enhance our understanding of genetic, physiological, nutritional, and behavioral characteristics of economic pests.

Recommendations: Encourage and support the development of performance standards for well-

established, standardized insect colonies. Promote the use of performance standards in evaluating insect consistency in standard tests of toxicity and activity.

4. Continuing increases in the use of laboratory-reared insects for research and methods development necessitates closer communication between the insectary (producer) and scientist (user). Routinely, producers supply insects as a service, but they usually do not convey changes in rearing procedures (such as dietary substitutes and changes in environmental regime). Generally, users are unaware of the effects such changes may have on their research, so they do not request such information. And the user seldom tells the producer about any changes that may have been observed.

Recommendations: Support and encourage the development of procedures for the exchange of appropriate information between the insect producer and the user. Scientists using laboratory-reared insects should be encouraged to test for and document insect quality as a prerequisite to the conduct of research.

5. The practical feasibility of using biological-control agents (chiefly insects, mites, and diseases) for control of many insect pests has been amply demonstrated. But large-scale rearing and use of many potentially effective entomophagous arthropods is prohibited by the cost and difficulty of rearing both host and agent. A concentrated effort is needed to develop artificial diets and in vitro rearing systems for entomophages.

Recommendations: Give high priority to the investigation and development of laboratory techniques for the in vitro production of entomophages. Studies and evaluation processes necessary for determining the competitiveness of laboratory-reared species should also be done.

Management of Insect-Rearing Systems

1. Rearing of biologically fit insects has become increasingly sophisticated and therefore requires local knowledge in areas such as the nutritional requirements of insects, disease recognition and prevention, and operation of automated equipment. Finding people with experience in these areas of insect rearing is difficult; most are trained on the job.

Recommendation: Recognize the need for skilled persons to rear insects, and encourage universities to develop formal courses that would prepare individuals for careers in this field. Such a program would have a broad curriculum in entomology, nutrition, insect behavior, ecology, pathology, and physiology.

2. Large-scale programs for rearing insects require extensive management if the products are to be suitable for their intended application. The facility must be maintained, ample supplies made available, the required number of insects produced on schedule, and personnel trained. And the results of research that improves rearing techniques must be integrated into existing programs.

Recommendations: Recognize that large-scale rearing programs require professional managers, and develop criteria and requirements for such positions. Encourage universities to develop curriculums to prepare individuals for careers in insect-rearing management by providing up-to-date advice about the needs of these positions. In the interim, plan workshops for managers of insect-rearing facilities to discuss mutual problems and their solutions.

3. The increased number of rearing facilities and greater quantities of insects being produced have exposed more people to potential health hazards associated with arthropods and their byproducts. These hazards may be either physical discomfort or some type of allergic reaction. This problem is only beginning to be recognized.

Recommendations: Identify and catalog specific health hazards associated with arthropods. Develop procedures and equipment to protect workers, and improve insectary designs and environmental controls.

4. Optimization of insect rearing requires the capability of retrieving and analyzing extensive data on many variables.

Recommendation: Implement available technology for systems analysis to facilitate the collection, analysis, and recall of essential data in programs for insect production and use.

Section 1

Establishment and Maintenance of Insect Colonies Through Genetic Control

The science and art of insect rearing has increased in complexity and sophistication as the need has grown for insects in entomological research and pest control. Early entomologists could perform elegant studies using insects collected from the field; or they could rear enough insects on natural hosts. But new concepts, such as the sterile-insect method or the mass production of parasites and predators, require many more insects. So artificial diets and environments have been developed. These new techniques are so successful that even further demands have been made on the insect-rearing specialist. Not only do entomologists want an abundant supply, but they insist that the insects function normally when released into their natural environment.

Unfortunately, in some cases the released insects do not function normally. Explanations for these malfunctions have been sought, and two biologically logical culprits found: pathogenic contamination and genetic deterioration. Microscopic examination will confirm or deny the presence of pathogenic organisms in insect cultures quickly; though eliminating the pathogens is not easy. But the causes of genetic deterioration are not so readily identified. Only detailed genetic studies covering two or more generations can determine the presence or absence of detrimental genetic traits and then only if such traits can be defined and measured. Even so, many authors have suggested remedial measures for assumed genetic deterioration.

I believe an unappreciated element of this problem is that the genetic changes taking place when an insect colony is started are natural ones that occur whenever any biological organism goes from one environment to another. These processes have been well studied as evolutionary events and involve such concepts as colonization, selection, genetic drift, effective population numbers, migration, genetic revolutions, and domestication theory.

In this section of the conference, we have tried to integrate some of these genetic concepts into our pool of knowledge on insect rearing. First, I discuss what happens to genetic variability in the process of domestication. What factors might change it, and which ones might be expected to have little or no effect? Then, assuming that we can define the type of insect we want, how can we change an insect population to conform to our expectations? We can use artificial selection; Anita Collins reviews this well-documented field. Finally, Dennis Joslyn discusses various strategies for maintaining genetic variability in reared insects.

Our conclusions in this section are encouraging even though changes in genetic variability during domestication of an insect population will be unavoidable. We must accept the inevitability of these changes if we want to rear insects in artificial environments. But, within budgetary restraints and the limitations dictated by the intended uses of the domesticated strains, those genetic changes need not ruin the program. We do, however, need to carefully study the type of variation present in the base populations and monitor changes that occur over time in the laboratory. For example, we can measure isozyme variation, chromosomal variation, visible markers, behavioral variants, body weight and size, reproductive traits, visual acuity, diapause response, and various types of activity. If we observe genetic changes over time, and such changes appear to be correlated with reduced performance of the laboratory strain, then correction can be made either by modifying procedures or by increasing the genetic variability of the colony. These corrections will require compromises between demands of the rearing program and the availability of appropriate genetic techniques. Such compromises are possible only if the insect-rearing specialist and the insect geneticist understand each other's problems and needs.

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Genetic Changes During Insect Domestication

By Alan C. Bartlett¹

Introduction

The development of artificial diets and mass-rearing techniques for insects (see Chambers 1977 for a definition of mass rearing) has progressed rapidly during the past 20 years. Most advances have been empirical; success has been measured in numbers of insects available for release per unit of cost (Gast 1968). But, having been successful in quantity, researchers are now examining insect quality (Huettel 1976, Chambers 1977), behavior (Boller 1972), and genetics (Mackauer 1973, McDonald 1976). This examination is continuing as we become more confident of our ability to mass-produce insects, and we now desire to have success measured in quality as well as quantity per unit of cost. Since most entomologists accept the need for mass-reared insects for the implementation of many insect-control techniques (see Knipling 1966 for an early discussion of the uses for mass-reared insects), the biological effects of laboratory production of insects must be examined in detail. This paper explores some of the genetic changes that may take place in the first generations of an insect population during its establishment in the laboratory.

The process of establishing and maintaining insect colonies has been reviewed by several investigators (for example, Boller 1972, 1979; Mackauer 1972; Davidson 1974; Hoy 1976; Huettel 1976; and McDonald 1976). In general, they have considered the process as a colonizing episode for the species, as a process of domestication, or as an expression of the founder principle of species evolution. These concepts are interrelated and their subtle distinctions need not be pointed out here. Instead, I will limit this discussion to the process of domestication, because it is the only concept that requires human participation. I will consider only those species that reproduce sexually. And I will not consider stocks that have been subjected to extreme selection pressures and used for very specialized purposes.

Genetic Changes

Changes in variability

Many discussions of the processes that take place during the establishment of an insect colony maintain that we change the population's level of genetic variability by forcing insects through a bottleneck (see, for example, Boller 1972, 1979). Spurway (1955), in describing the forces that can change gene frequency during domestication of a species, called this process a winnowing, an analogy that is more accurate than the bottleneck comparison. The idea of a restrictive bottleneck that a strain must go through on its way to the laboratory does not necessarily conjure up a sense of sifting and sorting of suitable genotypes of an inanimate environment. But winnowing is just such a sifting and sorting. One can visualize the force of selection blowing through the falling insects and removing the chaff of unsuited genotypes; also, some suitable genotypes will bounce off the winnowing board and be lost from the population (fig. 1).

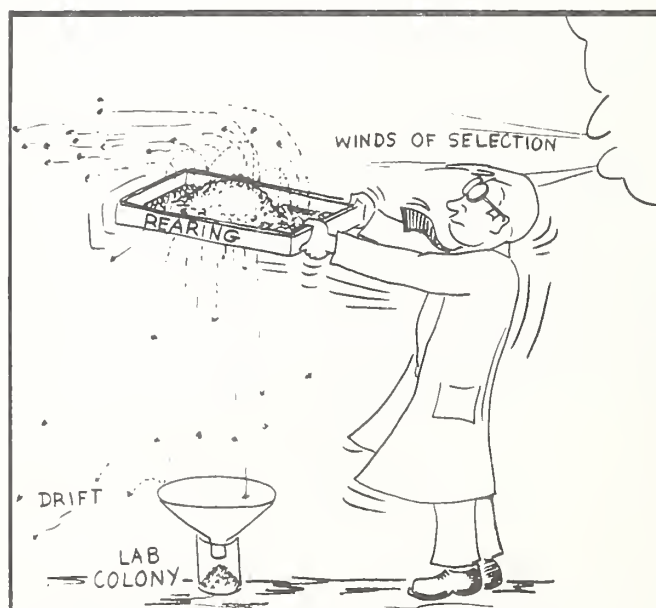


Figure 1.—Winnowing insects for laboratory domestication.

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Understanding the force of selection on existing genotypes and the random loss of genotypes is vital to any discussion of changes that take place in the domestication of a species. We have learned much about the processes of natural selection through studies of artificial selection (see "Artificial Selection of Desired Characteristics," by Anita M. Collins). Here, I will emphasize how natural selection can change a laboratory population's genetic variability. Lerner (1958) listed and discussed some factors that should be considered in an experiment in artificial selection. With some minor modifications, his list is given below as an outline of changes that a field-collected strain may undergo when introduced into the laboratory.

1. Laboratory and experimental populations are usually maintained in reasonably constant environments that are drastically different from those where earlier selection for stable developmental patterns has occurred. They are shielded from environmental fluctuations of various kinds and from predators; they are provided with shelter and food. In nature, selection may operate much more strongly in favor of individuals able to overcome unexpected stresses. A constant environment does not merely change the criteria that determine fitness; it may also greatly modify the whole genetic system of an artificially colonized population.
2. Interspecific competition is part of natural selection but is seldom involved in artificial colonization. Clearly, expensive rearing supplies should not be used for rearing competitors or parasites to produce interspecific competition in the laboratory; but we should also realize that genetic variability may change without this factor.
3. Members of natural populations can choose their own environments from those available. For domesticated organisms, microenvironmental conditions are generally made suitable for the average, or sometimes the poorest, genotype. Within the population, all the individuals are generally confined to the same environment.

Laboratory conditions will also cause other changes not directly related to the environment:

1. Density-dependent behaviors may be profoundly affected in laboratory conditions. For instance, mate-searching behavior is probably restricted in the small mating cages usually provided in the laboratory. Female egg-laying behavior probably changes when few deposition sites are provided. Apparently, the more finicky the species, the more behavioral changes will be necessary to adapt to the new conditions.

2. Mate-selection processes may be changed because unmated or previously mated females will have restricted means of escape. Promiscuous sexual behavior is an advantage in domesticated species, as is the ability or willingness to breed in confinement.
3. Dispersal characteristics, specifically adult flight response and larval dispersal, may be severely restricted by laboratory rearing conditions (Bush et al. 1976).

Kinds of change

Huettel (1976) discussed many of these possible changes in laboratory populations and suggested methods for monitoring and compensating for them. Some of these factors should also be considered in predicting the kinds and amount of changes an insect colony may experience. The first factor is the amount of variability present in the population. If a trait does not vary, then no genetic change is possible without mutation. But past experience suggests that most measurable traits are variable. In fact, recent work has disclosed an extraordinary amount of variation in native populations even in marginal areas (Prakash 1973); and, in inbred populations, high amounts can remain (Yamazaki 1972). So we may conclude that variability for many, or even most, important traits will be present in stocks collected from the wild.

What will happen to that variability in the process of domestication? We can take only a small part of the whole population to start our culture, which will now be a closed population. In an open population, the available genetic variation is both greater and of a different kind because of gene migration and environmental diversity in both time and space (Mayr 1970). In a closed population, the common alleles originally present will be represented, while rare alleles are likely to be missing. All genetic changes will be made from the limited genetic variation present in the original founders.

The number of colonizing insects will directly affect how much variation will be taken from the native gene pool. The lower the number of insects, the lower the number of represented alleles and consequently the greater the deviation of the mean phenotype of the colonized population from the mean phenotype of the parental population. In an interesting experiment reported by Dobzhansky and Pavlovsky (1957), in 17 months, lines founded with 20 individuals diverged from each other and from the original population far more than lines founded with 2,000 individuals. But even those lines founded by 2,000 individuals diverged from each other by as much as 50%. Powell and Richmond (1974) observed similar results for populations started with 6 and 300 chromosomes. The forces of natural selection imposed on a recently isolated gene pool may also cause significant changes in fitness

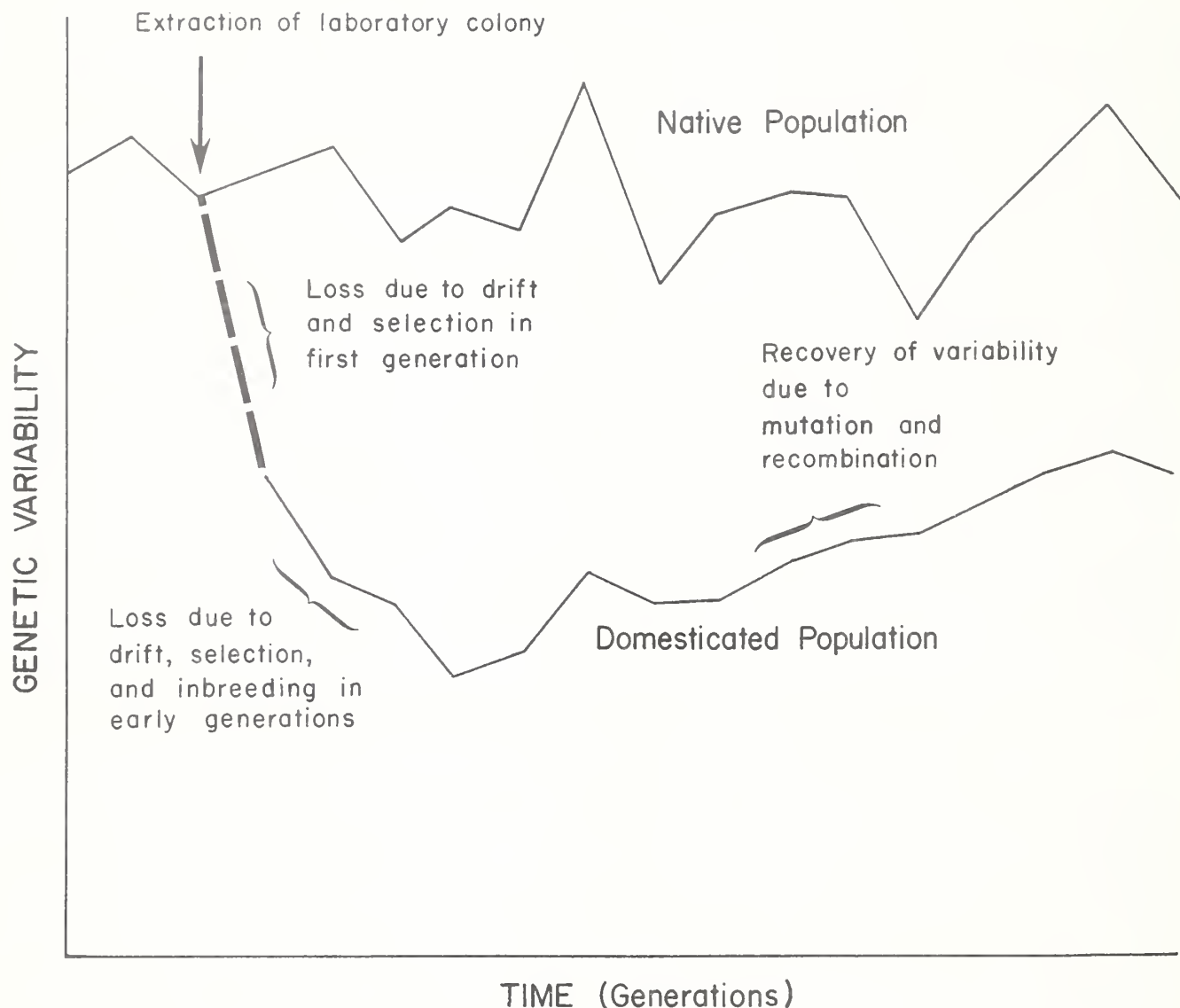


Figure 2.—A conceptualization of the changes in genetic variability that may take place during domestication of an insect species.

and produce what Mayr (1970) has called a genetic revolution. Such changes are illustrated in figure 2. In a laboratory environment, genes that have been at an advantage may now have some disadvantages (or, conversely, disadvantages may become advantages). For example, in northern latitudes the ability to diapause is useful to many insect species, while in the laboratory it may be neutral or even harmful. Similarly, behavior such as "startle response" or the ability to react to low concentrations of pheromone may change from adaptive to non-adaptive.

Lopez-Fanjul and Hill (1973a, 1973b) have described a series of experiments illustrating the patterns of genetic variability in laboratory populations. Each of three lines of *Drosophila melanogaster* Meigen that had been laboratory-reared for many years and one that was newly colonized was crossed to a strain that had been selected for high sternoplural-bristle number. Artificial selection was then practiced on the resulting hybrid lines. The three long-term laboratory strains were segregating for two similar alleles at loci controlling sternoplural-bristle number and for seven loci controlling enzymes. And the

newly isolated population contained some variation not present among those in the laboratory. But Lopez-Fanjul and Hill concluded that all the strains probably responded similarly to maintenance in the cages.

Such results support the theory that similar environmental conditions will select for similar genetic responses if not for identical genetic constitutions. After the transfer of a population from the field to the laboratory, a few individuals will be able to produce more offspring no matter how able they were in the field. Existing genic-balance systems that maintain homeostasis in the field may not confer fitness in the laboratory; so new balanced gene systems will be selected. If conditions remain the same in the laboratory, any later introduction of native genes will be subjected to the same process of genetic revolution; balanced gene complexes will evolve that will act the same as those previously selected and may, in fact, be the same (Lopez-Fanjul and Hill 1973b).

Introducing native genes to laboratory colonies

It has been suggested that a cure for genetic differentiation of laboratory colonies from native populations is regular introduction of native individuals. But there is a danger that genetic differentiation of the colony because of adaptation to laboratory conditions may lead to genetic isolation of native and laboratory populations. Oliver (1972), studying incompatibility in intraspecific crosses of Lepidoptera, found that geographically isolated races generally show heterotic vigor in the development of F_1 hybrids and a decline in fertility, skewing of sex ratio, and developmental abnormalities in backcross and F_2 progenies. Other studies, such as Jaenson (1978) and Jansson (1978), have found that the strength of genetic incompatibilities seems to be proportional to the differences between environments where the races occur. So there is a positive correlation between geographic distance and incompatibility of races. A similar positive correlation exists between the length of time two populations have been separated and their incompatibility. Introduction of native genes into a laboratory population should be regular—every one or two generations; it should not be delayed until problems become apparent (Richerson and Cameron 1974, Raulston et al. 1976, Sharp 1976). One hazard of introducing native insects into a colony is that they may never adapt and may therefore fail to produce enough progeny to continue the strain. Also, parasites or pathogenic micro-organisms could be introduced with the field-collected insects.

We can measure the effect of introducing native genes into a laboratory population by the simple formula for one locus (from Pirchner 1969) that

$$\Delta p = m(P_N - P_L),$$

where Δp = change in gene frequency for a given allele at a given locus,
 m = ratio of introduced individuals to individuals in the laboratory population,
 P_N = frequency of the allele in the laboratory population,
 and P_L = frequency of the allele in the laboratory population.

So the change in gene frequency will depend on the proportion of immigrant to native alleles and on the differences in gene frequencies between the two populations. If $P_N = P_L$, then no change in gene frequency will take place no matter how many native individuals are introduced. If the frequencies in the two populations are different, then the direction of change will be towards the highest gene frequency. For example, if selection has operated on the frequency of an allele to increase it over that in the native population, then the change will be in the direction of the laboratory strain. So the forces of selection and of immigration of native alleles will be antagonistic. The amount and direction of change will depend on the relative strengths of the two forces. If laboratory conditions remain constant, selection will reestablish the original gene frequency once native alleles are no longer inserted into the colony.

Changes related to the size of the breeding population

The genotypic structure of populations can also be affected by their breeding system. When mating partners are related to each other more closely than randomly picked individuals, the mating system is called inbreeding; outbreeding occurs when they are less closely related. Inbreeding implies mating of relatives and the production of progeny more homozygous than would be expected with random mating (panmixis). These homozygous individuals often exhibit harmful traits. The proportion of heterozygous individuals is decreased by $2pqF$ each generation (where p and q are the frequencies of alleles at a given locus and F is the inbreeding coefficient of the population). The inbreeding coefficient is directly related to the size of the breeding population. Therefore, the change in the inbreeding coefficient over one generation is estimated as

$$\Delta F = N_m + N_f / 8N_m N_f$$

where N_m = the effective number of breeding males,
 and N_f = the effective number of breeding females.

If any type of selection, artificial or natural, is present during the reproductive phase of a population, then the

rate of inbreeding will increase; that is, the population may be propagated by only a few individuals. The frequency of genes identical by descent will be increased in those families most favored by the selection process. The process of inbreeding alone will not change gene frequencies, only genotypic frequencies. But inbreeding plus selection, a condition often encountered in the domestication process, will have definite and rapid effects.

Measuring genetic changes

Electrophoresis could be used to measure changes in genotypes and gene frequencies against theoretic changes expected from combined selection and inbreeding. Falconer (1960) defined the panmictic (random mating) index (P), which complements the inbreeding coefficient ($P=1-F$), as the ratio of the frequency of heterozygotes at any time to the frequency of heterozygotes in the base population. So, if we could measure the frequency of heterozygotes for a pair of alleles in the base population (H_0) and of heterozygotes at that same locus at any one time, then the panmictic index for that locus at time (t) would be

$$P_t = H_t / H_0.$$

If we could measure heterozygosity at many isozyme loci, we could calculate the average heterozygosity over all examined loci (n) as

$$P_t = \sum_{i=1}^n H_{ti} / \sum_{i=1}^n H_{0i}.$$

The inbreeding coefficient would be $F_t = 1 - P_t$. How changes in gene frequency at the enzyme loci are related to changes at other loci (those affecting reproductive fitness, competitiveness, etc.) is currently being studied with electrophoresis. Whatever the outcome of these studies, it is already obvious that inbreeding and selection acting on the whole genome should be correlated with changes at specific isozyme loci.

Changes in reproductive ability

The most discussed effect of inbreeding is the reduction in reproductive performance of the inbred population. Changes in certain types of performance have also been suspected as a consequence of inbreeding depression by Davidson (1974) and Robinson (1977). Craig (1964) and Crystal (1967) show that this potential inbreeding can be counteracted with outbreeding, which will produce new variability and heterosis. Generally, native genetic material is incorporated into the laboratory-reared population before release, although Craig (1964) advocated keeping two colonies and crossing them. In fact, Young

et al. (1975) used this method effectively for the corn earworm, *Heliothis zea* (Boddie), when the first generation of laboratory insects was crossed with the native population.

Outbreeding seems to offer promise for improvement of laboratory populations, but theory suggests that only small increases in genetic variation could be expected (Falconer 1960). Heterosis depends, among other things, on differences in frequencies of genes that affect the traits in question. If two populations adapted to different environments are crossed, the outbred individuals may be adapted to neither of the original environments. So, when we consider using outbreeding for colony improvement, we must carefully choose the strains to be crossed. And such a technique may not be successful in every case.

Conclusion

An insect species that is taken from one set of environmental conditions and placed into another cannot survive unless the population can adapt to the new environment. Preadaptation or prospective adaptations can be refined or extended by imposed selection pressures. But, at first, existing adaptabilities enable the transition (Hansen 1977). This transition is a winnowing process with change and selection producing a domesticated strain. Such a strain must be adapted to the laboratory but may or may not be well adapted to the original field conditions.

During domestication, we should minimize the important changes and maximize reproduction. Since those changes that affect interaction of domesticated and native strains are important in most experiments and in control programs, the species' biology and behavior must be studied well (see Boller 1979 and Bush 1979 for further discussion of the types of biological studies that can be made). Given enough information on the biology and behavior of the species, we can adjust the laboratory environment to compensate for many of these important genetic changes. We may have to adjust our rearing procedures or our expectations of the mass-rearing colony, but such compromises should be based on known biological factors, not on conjecture. Within the limits imposed by budget, facility, and manpower, the following criteria should be considered when appropriate to the species: (1) Recognize that the effective number of parents will be much lower than the number of founder individuals, and try to compensate by starting with large founding populations (LaChance 1979); (2) compensate for density-dependent phenomena by using large mating cages, airflow to remove pheromone accumulations, more egg-laying sites than are needed, female hiding places, induced flight behavior, etc. (McDonald 1976); (3) adjust or maintain rearing densities to produce a proper balance of competition but not overcrowding (Sokal and Sullivan 1963, Peters and Barbosa 1977); (4) set environmental condi-

tions for the best, not the worst or average, genotype, and use fluctuating temperatures and photoperiods in all phases of the rearing environment; (5) maintain separate laboratory strains under unique conditions and cross these systematically to increase F_1 variability (Craig 1964, Roberts 1974); (6) measure frequencies of biochemical and morphological markers in founder populations, and monitor changes in gene frequencies over time (Bush and Neck 1976, Novy 1978, Bush 1979); (7) develop morphological and biochemical genetic markers for population studies and for marking the released strain so that a genetic-control technique can be evaluated effectively (Bartlett 1967, 1982; Huettel 1979); and (8) determine the standards that apply to the intended use of the insects, and then adapt rearing procedures to maximize those values in the domesticated strain (Boller 1979).

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Artificial Selection of Desired Characteristics in Insects

By Anita M. Collins¹

Introduction

Artificial selection is the alteration over time of phenotypic characteristics of a population of organisms through the intervention of man. Several major texts deal generally with theoretical and actual application of the science of genetics to artificial selection (see Lush 1945, Mather 1949, Falconer 1960, Li 1968, and Crow and Kimura 1970). Articles on artificial selection for insects include many on *Drosophila* spp. (see Robertson and Reeve 1957, Clayton et al. 1957, Madalena and Robertson 1975, and Frankham 1977) and on *Tribolium* spp. (see Wilson et al. 1965, Enfield et al. 1969, and Berger and Freeman 1974). Reviews of the application of artificial selection to insect populations (Bell 1963, Mackauer 1972, and Hoy 1976) have all held that it has great potential. Artificial selection has been used successfully for some time on two important domesticated insects—the silkworm, *Bombyx* spp. (Aizawa et al. 1961, Yokoyama 1979), and the honey bee, *Apis mellifera* Linnaeus (Rothenbuhler 1958, 1979; Rothenbuhler et al. 1968; Kerr 1974; Cale and Rothenbuhler 1975; and Goncalves and Stort 1978). Attempts have also been made to improve various insect parasites for use in pest control (Wilkes 1947; DeBach 1958, 1964; Hoy 1979; and Roush 1979).

A selection program must begin with basic information about the organism and the traits to be selected. Some understanding of the creature's reproductive strategies is necessary, including such things as how many males mate with each female, whether a male can mate more than once, how many young are produced at one time, and what interrelationships exist among those offspring. Theories about artificial selection in animals deal mainly with mammalian-type systems—a single mating (one male \times one female), few offspring at one time, diploid inheritance in both parents, etc. The theories must be modified, then, for insects like the social, multiple-mating, haplo-diploid honey bee. In any case, one must have a clear idea of what characters are to be selected and exactly how they will be measured. Knowledge of how the trait is inherited—whether it is controlled by one, a few, or many genes, will profoundly affect how it will be selected. Esti-

mates of a genetic parameter called heritability will predict whether the trait will respond to selection, by how much, and how fast. Given this basic biological information, one can choose a breeding plan and its selection criteria intelligently.

Desired Characteristics

Measurement

Many characters have been modified by selection. Morphological traits such as body weight (Enfield 1972) and thorax or wing length (Robertson and Reeve 1952) may be measured easily. Time of pupation (Englert and Bell 1970), temperature adaptation (White et al. 1970), disease resistance (Aizawa et al. 1961), DDT² resistance (King 1954, Robertson 1957), and sex ratio (Simmonds 1947), all physiological traits, may require more complex assessment. Evaluating behavioral traits such as host preference (Allen 1954), pollen collection (Mackensen and Nye 1966), geotaxis (Erlenmeyer-Kimling et al. 1962), phototaxis (Choo 1975a), dispersal (Ogden 1970), walking (Choo 1975c), and mating behavior (Manning 1968, Eoff 1977) may also be rather complicated. The special aspects of behavior genetics have been considered by Hirsch (1967), Dobzhansky (1972), McClearn (1973), Ehrman and Parsons (1976), and Fuller and Thompson (1978); and Boller (1979) discusses the special application of behavior genetics to insect rearing.

Some traits may be measurable only on certain individuals of a population or at certain times. For example, some traits such as egg-laying capacity, may be sex limited, or they may simply be expressed differently in each sex (Enfield et al. 1975). Other traits may require the organism's death before measurements can be made; so evaluation must be based on its closest relatives. In these cases, tests are conducted with progeny or sibs—individuals from the same litter or egg hatch of the same parents—leaving some alive and sacrificing others for measurement purposes. A familiar example of this form of study exists in the dairy industry, where bulls are evaluated on the basis of their offspring from many dams and used for more intensive breeding only if they are selected as sires. In insects, which usually have distinct

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²1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane.

life stages or castes, a particular character may have to be measured during one stage (Tucker 1980). The stinging behavior in worker honey bees, for instance, is not expressed by queens and drones. And finally, many behaviors in social insects reflect the activity of a group rather than of an individual. Honey production is an activity carried out by an entire honey bee colony; so the colony becomes the experimental organism (Rothenbuhler 1960).

Mode of inheritance

Once a technique for measuring a particular trait has been devised, understanding how the trait is inherited may be useful. The two major categories of traits are discrete, or qualitative, and continuous, or quantitative. With discrete traits, such as number of hairs and eye and body color, there may be one or only a few major genes controlling its expression. In these instances, relatively simple relationships exist among the several states of the trait (for example, brown eye color—the wild type—is dominant over the mutant scarlet). The dominant brown phenotype will be expressed if there are one or two brown alleles present. The recessive scarlet phenotype is expressed only when the organism is homozygous, carrying two scarlet alleles. There are also cases when incomplete dominance occurs, and crosses between discrete phenotypes will yield an intermediate phenotypic expression. An inheritance pattern of the discrete type may vastly simplify the selection procedure, perhaps even enabling complete fixation of one expression in one generation. Falconer (1960) discusses implications of simple modes of inheritance; more detailed discussions of the genetic analysis of discrete traits are in general genetics texts such as Strickberger (1968) or Merrell (1975).

Most characters that interest insect breeders will be quantitative traits. For characters like DDT resistance (Pielou and Glasser 1952), body weight (Bartlett et al. 1966), and mating behavior (Manning and Hirsch 1971, Kraemer and Kessler 1975), many genes are influencing the phenotypic expression of the trait, and clear-cut dominance relationships and modes of inheritance are not seen. Because quantitative traits are more complex and more common than qualitative traits, my discussion of artificial selection deals mainly with quantitatively inherited characters.

Effect of environment

The phenotype, or observable properties, of an organism is what is being measured. The phenotype is the result of interactions between the genotype, or genetic makeup, of the organism, and the environment that it lives in. For selection to occur, the phenotype must have some variation that can be attributed to the genotype. If all varia-

tion of the phenotype is caused by environmental conditions, then selection cannot be successful. Therefore, studies need to be conducted with a controlled environment that will reduce environmental variation during measurement of a phenotype. Measurements may have to be made in precisely controlled environmental chambers with the insects at a constant temperature and humidity, on the same food source, measured at the same age, and so forth. The more closely all environmental conditions that might affect the character can be controlled, the more accurate will be the assessment of the genotypic variation present in the population. The effects of environment on heritability and selection response are discussed by Falconer (1952) and Rendel and Binet (1974).

The type of character being measured will also influence the amount of environmental variation affecting the expression. Morphological and physiological traits are less open to environmental influence during development and expression than are behavioral traits.

Measured phenotypic variance, V_p , can be divided into several components. The first is the genetic variance, V_G , which itself can be divided into additive genetic variance, V_A , dominance deviation, V_D , and interaction deviation, V_I . All other variation is environmental, V_E , and is considered beyond experimental control. So

$$V_p = V_A + V_D + V_I + V_E.$$

Of the three types of genetic variance, V_D and V_I are generally considered to be less important than V_A . V_D arises from the property of dominance among the alleles making up a genotype; and V_I , usually rather small, is generally treated as a negligible complication. The breeding value of the organism, V_A , can be measured relatively easily in several ways and expressed as a ratio of additive genetic variance to total phenotypic variance. This ratio is the estimated heritability. The assumption made here, that environmental deviations and genotypic values are independent of each other, is not entirely true. Kulinčević and Rothenbuhler (1975), for example, found that susceptibility to disease varied with the virulence of the pathogen. One way this complication can be overcome is by specifying that the genotype be measured under specific conditions. Another assumption that is not always justifiable is that a specific environmental difference has the same effect on different genotypes (Bray et al. 1962, McNary and Bell 1962, Jinks and Connolly 1975). But comparing genotypes under favorable and unfavorable conditions may show that a genotype that does best under one set of conditions may not have the greatest yield or fitness under another. This variance of interaction between genotype and environment, usually regarded as part of the environmental variation, should not be overlooked in measurement of a characteristic, par-

ticularly when the same species is studied in different habitats (Druger 1962, Nye and Mackensen 1970).

Heritability

One of the most important properties of a quantitative trait is its heritability, h^2 , the ratio of additive genetic variance to the total or phenotypic variance;

$$h^2 = V_A/V_P,$$

that is, the proportion of total variance attributable to additive effects, which are the average effects of all genes affecting a character. The size of h^2 indicates how alike related organisms are. The most important function of h^2 in the genetic study of quantitative traits is that it can predict how reliable the phenotypic value is as a guide to the organism's actual breeding value. So heritability is a measurement of the proportion of the phenotypic variation that is attributable to genetic causes amenable to selection.

The value of heritability ranges from 0 (no genotypic influence on the variation of the trait) to 1 (all variation of the trait is genetically produced). Traits that are closely connected to reproductive fitness generally have low heritabilities. For example, values for litter size, egg production, egg-laying rate, and ovary size range from 0.1 to 0.3. Another reason for a low heritability value is inefficient measurement. If the technique does not accurately measure the desired trait, environmental variance, V_E , may be increased considerably; the proportion resulting from additive genetic causes would be reduced; and heritability would be decreased. Higher h^2 -values are expected in characters less important to reproductive fitness such as coat color, patterns of spotting ($h^2=0.95$ in mice; Strickberger 1968), and spot number (McWhirter 1969). These traits may be controlled by one or just a few genes. As an example of h^2 -values, consider some traits of *Drosophila melanogaster* Meigen: abdominal bristle number, 0.5; body size, 0.4; ovary size, 0.3; and egg production, 0.2 (Falconer 1960).

Heritability is a property not only of a specific character but also of the population and of the environmental circumstances influencing measured individuals. Environmental variance depends on the conditions of culture or management of the organism—more variable conditions reduce the heritability, more uniform conditions increase it. Genetic components are influenced by gene frequencies in the population, and these may differ between populations because of their different histories. Small populations maintained for a long time become more genetically uniform than do large, randomly mating populations, and they show lower heritabilities.

The simplest way to evaluate heritability would be to measure a population of mixed genotypes and one of identical genotypes in several environments. The first population would provide an estimation of total phenotypic variance;

$$V_P = V_A + V_E.$$

The second would measure only environmental variance, because all genotypes would be identical. The difference between these two phenotypic variances would be the additive genetic value. Heritability could then be directly calculated from the ratio of additive genetic variance to total phenotypic variance.

The standard approaches to measuring heritability require comparing the merits of related individuals and estimating heritability from the covariance between them or from a regression or correlation coefficient. Estimates of heritability from covariance and correlation in domestic animals must consider a major environmental source of covariance, the maternal effects. But such influences as a common uterine and rearing environment for animals of the same litter or mother may not be particularly important in insects.

A straightforward method for estimating heritability is to use the regression of offspring on parent. The data, measurements of parents and the mean values of their offspring, are used to calculate a regression coefficient, b . If this is the regression of offspring on one parent, b_{op} , it is a valid measure of $\frac{1}{2}h^2$; if the regression is offspring on midparent (average of the two parents), b_{mp} , it actually measures h^2 . Examples of using this method for estimating heritability in insects are found in Enfield et al. (1966), Morris and Fulton (1970), and Wong and Boylan (1970).

Heritability is most often estimated by sib analysis. Each of several males (sires) is mated to several females (dams), and some offspring from each female are measured. The individuals measured form a population of half-sib and full-sib families. An analysis of variance is calculated to divide the phenotypic variance into components attributable to differences in sires, in dams mated to the same sires, and among offspring of the same female. The variance component from sires, dams, and the total must be calculated from the mean square values (table 1). The total variance, or phenotypic variance, is calculated because it is not necessarily equal to the observed variance as estimated from the total sum of squares, though the two seldom differ by much. With these values, estimates of heritability can be made from the sire component, the dam component, or a combination of the two (table 2).

Table 1.—Formulas for calculating components of phenotypic variance from analysis of variance mean squares (*MS*) for a population of sibs and half-sibs¹

Source of variance	Variance	Calculation ²
Between sires	σ^2_{sire}	$= MS_{sire} - MS_{dam} / dk$
Between dams	σ^2_{dam}	$= MS_{dam} - MS_{within} / k$
Within progenies	σ^2_{within}	$= MS_{within}$
Total population	σ^2_{total}	$= \sigma^2_{sire} + \sigma^2_{dam} + \sigma^2_{within}$

¹See also Falconer (1960).

²*d*=number of dams; *k*=number of offspring per dam.

Heritability can be estimated from the offspring-parent relationship in a population with the structure set up for sib analysis. For many domestic animals, however, such a population structure has few male parents, so the simple regression of offspring on one or the other of the parents is unsuitable. But heritability can be estimated from the average regression of offspring on dams—regressions are calculated for each group of dams mated to the same sire, and they are pooled to give a weighted average.

These methods of estimating heritability have been developed for use with diploid organisms. If the particular insect being evaluated is not diploid in both sexes, somewhat different forms may be required. For example, Rinderer (1977) modified sib analysis for the honey bee, a haplodiploid colonial organism; he also delineated several problems of estimating heritability in a haplodiploid social organism.

Artificial Selection

Selection methods

Selection of males and females with the desired characters to parent the next generation can be done in several ways (see Wright 1921, Mather 1941, and Hazel and Lush 1942). Single individuals can be chosen on the basis of their own phenotype (for example, the expression of a particular mutation) to be mated with a specific other individual. This is individual selection. In a variation on individual selection called mass selection, large numbers of selected individuals are put together en masse for mating, a common occurrence in rearing large numbers of insects.

Family selection is the choice of individuals based on the mean phenotype of the family that they come from. This method requires selection of the entire family for use as parents and is preferable for characters having low heritability. But the method is limited because all members of

Table 2.—Formulas for calculating heritabilities from phenotypic variances determined from analysis-of-variance mean squares for a population of sibs and half-sibs (table 1)¹

Heritability estimate	Calculation
h^2_{sire}	$4\sigma^2_{sire} / \sigma^2_{total}$
h^2_{dam}	$4\sigma^2_{dam} / \sigma^2_{total}$
$h^2_{combined}$	$2(\sigma^2_{sire} + \sigma^2_{dam}) / \sigma^2_{total}$

¹See also Falconer (1960).

the family will generally be reared in the same environment. One variation of family selection is sib selection, usually used for traits requiring the death of an organism, in which an individual's phenotypic value is based on measurements of its siblings. Another is progeny testing in which offspring are measured. If only the best individual from each family is chosen for mating, the method is referred to as within-family selection. This approach is desirable if a common environment, such as a shared uterus, has a major effect on the size of the environmental variance.

In all cases of selection by group, the calculations of h^2 and R (response to selection), differ from those used with individual selection (see Kojima and Kelleher 1963, Wilson et al. 1965, Berger and Freeman 1974, and Katz and Enfield 1977). For these calculations, an assumption is made that the generations are kept separate—individuals are selected from a base population, used to parent offspring (first selected generation), and only the offspring are chosen to parent the second selected generation. The generations would *not* be separate if selected individuals from the base population and the first generation were intermated to produce the second generation. For most insect-rearing procedures, generations will probably be discrete.

Response to selection

Selection will change the population mean of the selected character by an amount, R , the response to selection. A second parameter, selection differential, S , is the measure of average superiority of those individuals selected as parents over the total population. R and S (fig. 1) are related by the regression coefficient of the offspring on their parents, b_{op} . So

$$R/S = b_{op}, \text{ or } R = b_{op}S.$$

If there are no significant nongenetic causes of resem-

blance, such as maternal effects, and the selected phenotype is not correlated with general fertility and viability within the selected population, then R and S can be related directly to heritability. So

$$R = h^2 S.$$

The relationship of R , S , and h^2 is most useful for prediction. Once parents have been selected for production of the next generation, S will be known. The value of b_{op} , the regression coefficient, can be calculated from the previous generation; or an estimate of heritability can be made from the parental population. Selection does change the population, and these two values, b_{op} and h^2 , can change with the selection. Theoretically, then, the prediction is accurate only for a single generation. In practice, however, the predicted value of regression or heritability actually holds true over several generations (Falconer 1960).

There are two factors affecting the size of the selection differential, S . These are the proportion of the population selected to be parents and the phenotypic standard deviation of the trait being selected. If a small proportion of the population is selected for mating, that proportion will represent only the most extreme members of the population; their mean value, $\bar{X}_{P'_0}$, will be very different (high value of S) from the mean of the total base population, \bar{X}_{P_0} (fig. 1). But, if the proportion of parents is larger, many will have more intermediate values and the parental mean, $\bar{X}_{P'_0}$, will be less different (low value of S) from the total mean, \bar{X}_{P_0} . The phenotypic standard deviation, or variation, affects the size of S , the difference between selected extremes and the base population. With large

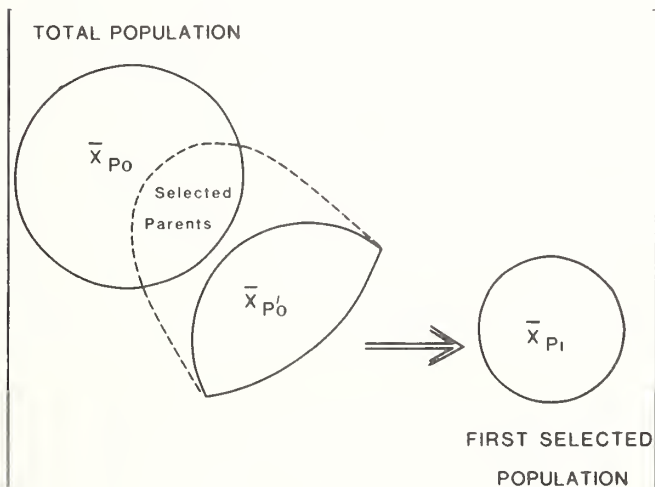


Figure 1.—Diagrammatic representation of response to selection, R , and selection differential, S . $R = \bar{X}_{P_1} - \bar{X}_{P_0}$, and $S = \bar{X}_{P'_0} - \bar{X}_{P_0}$.

variation, the extremes will be quite different from the average values for the trait, hence a high value of S . With little variation, the differences can be slight. The estimate of S for different traits and different populations is expressed in terms of σ_p (square root of V_p) to determine the quantity i , intensity of selection. So

$$S/\sigma_p = i, \text{ or } R = i\sigma_p h^2.$$

Manipulation of heritability, intensity of selection, or phenotypic variation can improve the rate of response, R . To increase heritability, one must decrease the environmental variance by manipulating rearing and measurement conditions. Decreasing proportion of individuals selected, and so increasing the intensity of selection, will have a similar effect. Of course, this decrease is limited by the size of the population being used for selection, which will be somewhere between the maximum number of organisms that can be reared and measured at any one time and those required to maintain a biologically functioning population. The selection intensity practiced per unit of time can also be increased by decreasing the generation interval or by maximizing the number of offspring produced in each generation. For honey bees, this increase might be several hundred colonies per year, while for *Drosophila* the number of measured units (flies) may be several orders of magnitude greater. Phenotypic variation is much less amenable than heritability or intensity of selection to manipulation because it is limited to what is biologically available. To manage phenotypic variation, a breeder should insure that the base population has maximal variation included in its members, or he should use crossbreeding during the selection program.

While artificial selection is being done, natural selection will also be affecting the population. Mainly, it will alter the fertility of the selected parents and the viability of the offspring (Wilkes 1947, Hiraizumi 1961, Kress et al. 1971). Using weighted values from the parents based on the different numbers of offspring that each group contributes to the succeeding generation, one can recalculate the selection differential. This quantity is the effective selection differential rather than the expected selection differential. If there is a large difference between these two calculated values of S , then this population is undergoing a great deal of natural selection.

There will be some variability in the population mean from generation to generation (fig. 2), mainly because of how the environment affects the expression and measurement of the phenotype. So, to be most precise, estimates of R need to be made over several generations. Such estimates are made by fitting a regression line to a series of generation means (Robertson and Reeve 1952, Englt and Bell 1970). The slope of this regression line then represents the best measure of the average response per

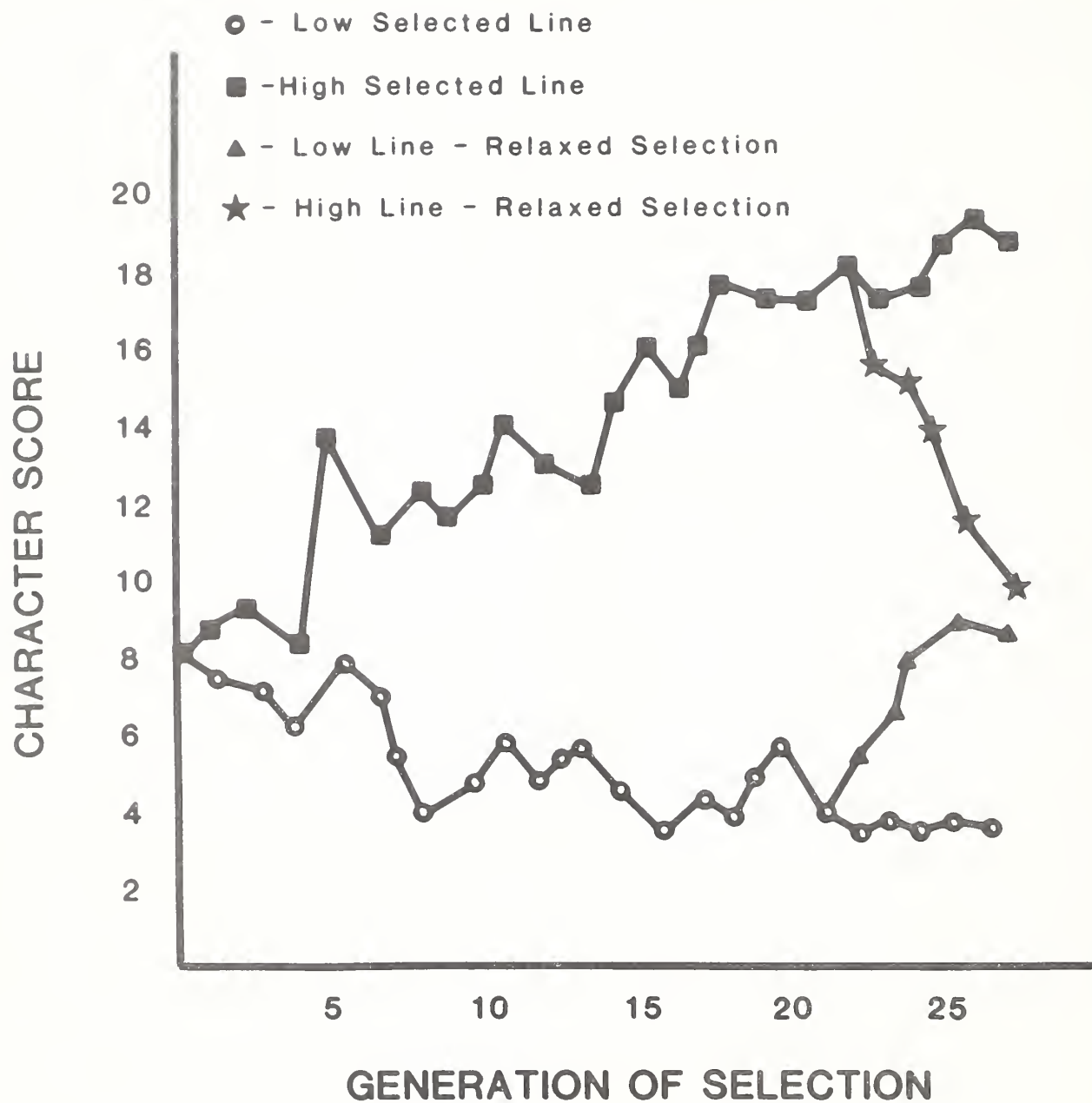


Figure 2.—Results in a hypothetical bidirectional selection program.

generation. If selection is bidirectional—for example, if one is selecting for high body weight in one population and low body weight in another—calculation of the response will be better than a comparison between a selected population and an unselected control; bidirectional selection gives a greater divergence of the two populations being compared.

Heritability can also be estimated as

$$h^2 = R/S.$$

If the value for S used in this calculation is the effective, or weighted, selection differential, it will eliminate the effects of natural selection and estimate heritability only on the basis of artificial selection. This realized heritability may not be the best estimate of the actual value of h^2 , but it is the most useful figure for comparing the effectiveness of different selection procedures, especially when different intensities or methods of selection are in use. Realized heritability is usually calculated as the slope of a regression line fitted to a plot of the generation means, R , by the cumulative value of the selection differential, S (Meyer and Enfield 1973, Choo 1975a).

Other considerations in selection

In the same way that generations differ, responses to selection may differ between different lines or populations undergoing selection (Defries and Touchberry 1961). Genetic parameters such as heritability and the regression of offspring on parents may also differ. If the selection is bidirectional, the response may be asymmetric (Englert and Bell 1970). That is, selection may proceed more rapidly in one direction than in the other (fig. 2). Falconer (1960) gives several possible causes for this variation in response.

The genetic structure of populations undergoing selection will change through generations. One of the effects of this change is that the size of the response does not remain at its initial level but becomes smaller with succeeding generations until the population reaches a plateau for the phenotype, the selection limit. In figure 2, for example, the low-selected population has reached a plateau at generation 20 because the desired alleles have been fixed (Bell et al. 1955, Brown and Bell 1961, McEnroe 1967). If further progress is desired, the population must be given greater variation through the introduction of other alleles. These new alleles can be introduced by crossing highly selected lines, by introducing environmental stress, by changing the selection criteria, by using radiation (Bartlett et al. 1966), or by outcrossing the selected population.

At the same time that selection for a desired trait is being carried out, there may be unintentional selection of correlated traits (Robertson and Reeve 1957, Hiraizumi 1961, Wong and Boylan 1970, Kress et al. 1971, Choo 1975b). Among the several causes for unintentional selection is pleiotropy—one gene influences more than one characteristic. Or several genes may be closely linked on

the chromosome, and selection for one gene will inadvertently carry along its linked associates. Conversely, beneficial combinations of several genes may have evolved together and be closely linked; though, if these linkages are broken up during selection procedures, the product might be less desirable or even harmful, phenotypes. Finally, traits may be correlated in some way because of common parts in those traits. For example, honey bees have a 0.5 correlation between the rate of hoarding sugar syrup in the comb and their response to alarm pheromones. So high hoarders are usually fast responders. This correlation may exist because response to a chemical stimulus (sugar or alarm pheromone) is necessary for both types of behavior (A. M. Collins and H. A. Sylvester, unpublished data).

A major consequence of continued selection, particularly in small populations, is inbreeding depression, a reduction in the reproductive capacity and physiological efficiency of the organisms undergoing selection. Hybrid vigor (heterosis) occurs when the fitness lost during inbreeding is restored after two inbred lines are crossed (Cale and Gowen 1956, Enfield et al. 1966). One selection method uses both inbreeding depression and hybrid vigor by inbreeding many lines selectively for several generations then crossing them to restore their fitness. Generally, this technique is effective only in closely related populations such as those reared in laboratories. Since widely different wild populations fail to show heterosis when crossed, each may be adapted to its own environment, and progeny produced by crossing them are adapted to neither (Falconer 1960).

In some organisms, breeding can have dramatic effects. In honey bees, for example, the system for sex determination is tied to homozygosity of a specific locus. Homozygous and, in the case of haploids, hemizygous bees are male. Heterozygous bees are female. Continued inbreeding rapidly increases homozygosity. Within a few generations, the probability that many of the diploid bees will also be homozygous and develop as males becomes quite high. As diploid males are generally destroyed by the workers, the colony rapidly deteriorates. If such a situation arises in organisms undergoing selection, the rate of inbreeding during selection must be kept low.

When lines are crossed to make use of hybrid vigor, some crosses will produce progeny that are more fit than those produced by others. This variation is due to the combining ability of each line. If the ability of a particular line to combine with several other lines is measured and a mean calculated, the result is the general combining ability. Specific combining ability is the measurement of increased vigor between two lines. One can choose the expression of combining ability by using a program called reciprocal recurrent selection (Kincaid and Touchberry 1970, McNew and Bell 1970). In this plan, the selection of parents in the lines is based on the performance of their progeny from crosses with another line. The best combining parents are then mated within their respective lines.

The selection index

In practice, the least effective way to select for several characters is to use tandem selection—to select for one trait at a time. Instead, an independent culling level can be established for each trait being selected, and all animals below this level for any trait are culled. This technique requires many more animals than are needed for tandem selection to have enough selected to carry on a viable population. And selection may be slowed if culling levels must be reduced to leave a viable breeding population. So the best way to select for several characteristics is to use a selection index, which combines all the phenotypic measurements into a single value. Each trait may be weighted according to its relative economic value, heritability, and correlation with other traits in the index. The result is one number that determines the culling level (Hazel 1943, Falconer 1957, Tallis 1962, Okada and Hardin 1970, and Yamada et al. 1975). Hoy (1979) gives an excellent flow chart showing the decisionmaking and experimental steps in developing such a program. Before attempting to develop an artificial selection program for a particular organism, one should review the literature on related organisms to discover if there may be special problems because of their specific biology.

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Maintenance of Genetic Variability in Reared Insects

By Dennis J. Joslyn¹

Introduction

Without genetic variability, populations of organisms cannot adapt to changing environments. In recent years, many studies have been done on the levels and maintenance of heterogeneity in natural insect populations (for reviews, see Lewontin 1974, Wagner and Selander 1974, Powell 1975, and Selander 1976). But there have been few studies on the genetics of laboratory-reared insects, particularly those being reared for use in nonchemical pest-control programs. A few attempts have been made to describe some of the genetic events occurring in laboratory-reared insects (Bush 1975, 1977; Sluss et al. 1978; Bartlett 1981; and Pashley and Proverbs 1981). But, even with few studies to verify it, researchers generally support the theory that mass-reared insects intended for release must undergo some genetic changes during colonization; the artificial environment alone is enough cause for such changes. To date, how these genetic changes affect the outcome of pest-control programs has not been determined.

One effect of colonization can be the reduction in genetic variability. This reduction could alter the ability of reared insects to fulfill their role in a pest-control program. So the adaptability of laboratory insects must be determined by monitoring changes in genetic content, and adequate levels of variability must be maintained.

Population geneticists today are debating what mechanisms cause natural populations to maintain high levels of genetic variability; the debate is often called the neutralist-selectionist controversy (Nei 1975, Kimura 1979). Discussions center on whether random processes (neutralists' view) or nonrandom events (selectionists' view) are primarily responsible for maintaining the high levels of variability in field material. The question is far from being answered; but apparently both processes are operating. In laboratory strains, the amount of variability and the mechanism of maintenance should be determined for each species, especially in mass-rearing programs. In conventional biological control, parasites are reared to

suppress the pest species (Mackauer 1976); in genetic control, the genetic apparatus of the pest is used to effect its own reduction. Both approaches aim to curb the reproductive potential and therefore the size of the target population by the release of large numbers of artificially reared insects; and, in both cases, variability permits the production of insects that will interact effectively with the wild type. Eventually, the control principle for which the insect was selected or engineered will spread throughout the native population via a series of timed releases. These releases use planned ratios of laboratory to wild insects that are based on estimates of the target population's density.

For any insect-rearing program, then, the purpose of maintaining genetic variability is to insure the production of competitive individuals that affect target populations as planned. So mating competitiveness is especially important in genetic-control strategies intended to transfer a sterility principle into the wild population. Also, predators produced for biological control must not lose their ability to seek out their hosts. Because the laboratory is an artificial habitat, artificial insects (biotypes or ecotypes) can be produced inadvertently during colonization, especially when the rearing environment does not vary and insects adapt to one set of conditions. Such environmental uniformity may produce enough release insects; but, if a less competitive organism results, the purpose of their rearing is thwarted. For example, in a program rearing the screwworm, *Cochliomyia hominivorax* (Coquerel), the flies were unintentionally selected for a variant of the flight-muscle enzyme, alpha-glycerophosphate dehydrogenase (Bush et al. 1976, Richardson 1978). These mutants could not transfer the sterility factor into the wild population because their diurnal flight activity differed from that of wild flies. Reproductive isolation had developed in the factory-reared flies because of directional selection.

Understanding genetic variability in laboratory colonies requires understanding the arrangement of genes; sources of variation; and the measurement, decay, and maintenance of heterogeneity. Understanding how genetic variability decays and is maintained in nature helps us understand how it decays and can be maintained in the laboratory. But laboratory insects live in a controlled, often unvarying environment; and the small size of laboratory colonies limits the gene pool. Therefore, while variability, gene arrangement, and sources of variation may be similar between laboratory and field populations,

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decay of variability is much more rapid in laboratory colonies. Then, for a successful control program, it is necessary to design appropriate measures that will offset such rapid genetic decay.

Arrangement of Genes

External and internal variability

The evolution of a species depends on changes in gene frequencies over time. During this process, new genes appear through mutation and increase in frequency in a population, while others decline or are eliminated. These changes are part of the mechanism of natural selection or differential reproduction and enable organisms to adapt to varying environments. Genetic variability is classified into two categories—external and internal—on the basis of phenotypic effects. Both help the evolving species to adapt. External variability includes both dominant and recessive morphological mutants and an assortment of behavioral, physiological, and ecological variants. In the heterozygous condition, recessive mutants, regardless of the nature of their phenotypic effects, remain hidden; they are a cryptic variability (Dobzhansky 1970, Lewontin 1974). Genetically regulated physiological variability is especially important to an insect-rearing program because of the associated reproductive effects. Lethal, semilethal, and sterility genes, for example, are built-in genetic loads that lower reproductive fitness. Rearing conditional mutants (those expressed only in specific conditions), such as those responsible for pesticide resistance, temperature sensitivity, and disease susceptibility, may severely restrict the diversity of the rearing environment. But a restrictive laboratory environment limits the adaptive potential of the reared insect and may reduce its effectiveness at release.

Internal variability comprises cellular and molecular genetic differences; studying it usually requires special methods of analysis. Currently, the forms of internal variability being most widely applied in population analysis are chromosomal and protein variation (Selander 1976, John 1981).

Many insect vector species are genetically polymorphic for both external and internal traits. In their review of the genetics of medically important arthropods, Spielman and Kitzmiller (1967) pointed out the extent of external variability in both field and laboratory populations. Although they considered the value of internal chromosomal variation to be important for analysis of population genetics, Spielman and Kitzmiller also cited pioneer studies on external variability in laboratory strains of such pests as *C. hominivorax*, by La Chance and Hopkins (1962); house fly, *Musca domestica* Linnaeus, by Sullivan

and Hiroyoshi (1960); German cockroach, *Blattella germanica* (Linnaeus), by Ross and Cochran (1965); scrub typhus chigger mite, *Trombicula akamushi* Brumpt, by Goksu et al. (1960); and yellowfever mosquito, *Aedes aegypti* (Linnaeus), by Craig et al. (1961). For *Ae. aegypti*, VandeHey (1964) relied on the inbreeding procedures (Dobzhansky 1970) long used for studying variation in *Drosophila* species. The procedures reveal the hidden variability contained in heterozygous individuals.

Another genetic technique adapted to analysis of insect pest populations from *Drosophila* studies has involved the use of giant chromosomes. In salivary glands of *Anopheles* mosquito larvae, polytene chromosomes have been used extensively to detect inversion polymorphisms (Kitzmiller et al. 1967; Davidson and Hunt 1973; Coluzzi and Kitzmiller 1975; Kitzmiller 1976, 1977; Coluzzi et al. 1979). Unfortunately, most pest species do not have these giant chromosomes. Recent developments in chromosome-banding techniques (Newton et al. 1974, Steiniger and Mukherjee 1975, Motara and Rai 1977, Mezzanotte et al. 1979) use mitotic and meiotic chromosomes to reveal heterochromatin variability in populations. These techniques, including C, G, and Q banding, should provide an alternate source of information about the organization of chromosome variability in insect pests.

Some of the methods now being used to characterize internal variability in populations stem from the field of molecular biology. One method, gel electrophoresis, has been particularly useful. By separating charged protein molecules in an electric field, it has permitted the identification and quantification of gene-enzyme products known as allozymes. One advantage of the technique is that gene products can be examined directly in an individual, and variability can be easily determined. Gene frequencies can be found rapidly for a large number of individuals, and shifts in allozyme content in a population can be detected for many environmental influences.

Although the value of genetics to insect rearing has been recognized (Craig 1964), only recently has electrophoresis been used for genetic analyses of mass-reared colonies or longstanding laboratory strains (see, for example, Bush et al. 1976, Munstermann 1979, Bartlett 1981, and Pashley and Proverbs 1981). Analyses of natural populations have shown that considerable variability exists at the allozyme level (Powell 1975, Selander 1976). Insects being propagated for release must contain comparable levels of variation and should genetically resemble as nearly as possible the population to be controlled. When levels of variability have declined, they should be restored before release. Or loss of variability can be prevented when the colony is established or when it is replenished at periodic intervals afterwards.

Genetic load

Not all variability is beneficial. Inbreeding, which is unavoidable in laboratory colonies, may elicit recessive alleles that reduce the reproductive fitness of the population. So these genes are a genetic load; they may have lethal or sterile effects. In a genetic-control program, such genes could be very useful as a source of reproductive incompatibility between laboratory and field stocks; in fact, they are important as control mechanisms (Davidson 1974, Pal and Whitten 1974). But, in general rearing programs, the goal is to provide enough healthy insects that can seek out wild insects. This goal requires that the natural genetic load of the species be low; in a genetic-control program, additional load or reduction in fitness in the form of sterility is induced at the time of release.

Countering genetic load is helpful to a laboratory colony. Much of the variability of heterozygous genotypes is potentially adaptive in appropriate circumstances. Such variability allows insects to adapt to changing environments. It is also highly desirable in a control program requiring that founding stock adapt to the laboratory quickly yet remain variable enough to be able to interact effectively with the target.

Sources of Genetic Variability

Mutation and adaptability

The ultimate source of all genetic variability is mutation, or hereditary change, in the genetic material itself. Whether or not a given mutant will be harmful depends on the genetic composition at other loci and on its surroundings. Mutations are random events that provide the variability for selection to act on so organisms can adapt to varying environments. Some mutations are not subject to selection. These are distinguishable from both the harmful (maladaptive) and beneficial (adaptive) types and are considered to be adaptively neutral. In *Drosophila pseudoobscura* Frolova, for example, Yamazaki (1971) found that laboratory flies carrying two different alleles for the *esterase-5* locus showed no differences in viability, development, or fecundity. Furthermore, that the starting frequencies of these genes remained the same for 2 years in experimental populations suggests that they were adaptively equivalent.

Since mutant genes do not exist alone, genetic background influences their fate. In comparing the adaptive value of radiation-induced mutants in *Drosophila melanogaster* (Meigen) in a homozygous background to their adaptive value in a heterozygous background, Wallace (1958, 1963) showed that a new mutation in a heterozygous condition was better for a fly if the rest of its genome was homozygous not heterozygous. Futuyma

(1979) suggested that such mutations could lead to enhanced adaptation either in populations that are highly inbred or in those in new environments. Both circumstances occur in insect-rearing programs.

Survival of mutants in insect colonies

Conditions that influence the spread of a newly arisen mutant in laboratory colonies were generally considered for diploid organisms by Kimura (1955, 1962). He found that, as laboratory colonies are finite populations, variability in reared insects has to depend, in part, on mutations being able to survive and increase in frequency. After the mutation rate, perhaps the greatest influence on the rate of a mutation's diffusion is population size. In the laboratory, population sizes fluctuate. During an expansion, a given mutation should increase in frequency. A beneficial mutant would increase rapidly under these conditions and eventually attain a fixed frequency; a neutral mutation would ultimately become extinct. But contractions of colonies should diminish the spread of new variants. Spiess (1977) recounted the probabilities for both the fixation and extinction of neutral alleles arising through mutations; for q , a single neutral mutant (one with no selection value) in a population having N individuals, the ultimate probability for fixation is q_0 or $1/(2N)$. The initial frequency of a neutral mutation is also $1/(2N)$. Since the frequency of the alternate allele, p , is p_0 or $1-q_0$, then the probability of extinction for mutation q is $1-[1/(2N)]$, since $p_0+q_0=1$. Usually, neutral alleles require a long time to become fixed, about $4N_e$ generations (Hartl 1980), where N_e is the effective population size or number of individuals actually contributing to the next generation's gene pool. In mass-rearing programs where the colony must be divided to facilitate the rearing of enough insects, many subpopulations may approach a size of 10,000 or more individuals. A neutral allele arising in a subpopulation of 10,000 members would have a very low probability of becoming established (1 chance out of 20,000) and would require at least 30,000–40,000 generations to become fixed.

Another factor influencing the rate of a mutant's spread in a colony is the generation time of the species. Shorter generation times result in higher numbers of mating pairs available to a colony during any given interval. For selectively adaptive mutants in particular, higher numbers of immediate descendants are more likely to receive a mutation if the parent generation has more mating pairs. Neutral and harmful mutations, however, would be eliminated eventually, regardless of population size or reproductive rate.

Genetic recombination

Most rearing programs involve sexually dimorphic species. Because of this separation of sexes into different individuals, reproduction requires the union of haploid gametes from each sex to form a diploid zygote during fertilization. This fundamental aspect of sexual reproduction affords another source of variability through genetic recombination. During meiosis, homologous chromosomes may exchange segments during pairing (synapsis) and crossing over. This reassociation of alleles amplifies genetic variability, producing new phenotypes. For example, in the experiments on *D. pseudoobscura* done by Dobzhansky and his coworkers on how recombination affects variation in viability, recombining advantageous alleles at different loci produced different viabilities in changing environments (Lewontin 1974). Though genetic recombination results in new gene assortments, not all arrangements may be beneficial. Often, a population will have blocks of coadapted genes (adapted to function together). If these blocks are disturbed by recombination, the fitness of the organism will change.

Measuring Heterogeneity

Heterozygosity and polymorphism

Heterozygosity and polymorphism are measurements that provide estimates of the amount of variation in a population. In classical genetics, heterozygosity occurs where one or more loci have dissimilar alleles. For homozygosity, alleles at a given locus are the same. But unexpectedly high heterogeneity has been found in natural populations, mainly through electrophoretic analysis of allozymes. So heterozygosity has now become an important measure of population variation. Two types of heterozygosity—individual (h) and population (H)—are now recognizable, and may provide useful information about the genetic profile of any population. The quantity h is defined as $1 - [\sum(x_i^2)]$, where x_i is the frequency of the i^{th} allele in a population (Selander 1976). The value of h is as a measurement of the average amount of variability that exists at a locus. The average h across all loci examined (N) is the value H defined as $\{1 - [\sum(x_i^2)]\}/N$. H is the most frequently used measure of heterozygosity and denotes an average overall level of variability in a population.

Polymorphism has been defined by Selander (1976) as the occurrence of two or more alleles at a locus with the least frequent not maintained by recurrent mutation alone. The frequency of the least common allele is usually set between 0.01 and 0.05. In practice, most levels of genetic polymorphism are determined from electrophoretic data. Allozyme separations allow calculation of gene frequencies directly from gels. The most meaningful measure of

polymorphism is the percentage of loci that are polymorphic (P). In natural populations of insects, this figure commonly runs as high as 50% or more. But H values for *Drosophila* species, for example, range from 10% to 20%.

Other measurements of variability that are commonly used have also developed from electrophoretic studies. The average number of alleles per locus provides one such estimate. In two studies of esterase isozymes in mosquitoes, for example, considerable variation has been found in natural populations. Saul et al. (1977) found as many as 14 codominant alleles at the *esterase-6* locus in *Ae. aegypti*. In the mottled wing *Anopheles*, *Anopheles punctipennis* (Say), the *esterase-A* and *esterase-B* loci (Narang and Kitzmiller 1971) have seven codominant alleles each. As more loci are studied in a population, the total number of electrophoretically distinguishable alleles can be determined and used as an estimate of overall heterogeneity. In both field and laboratory populations, as many (at least 25) different gene-enzyme systems should be assayed as possible to accurately estimate the amount of variation. Statistically reliable sample sizes are critical also. Lewontin (1974) suggests that at least 50 individuals be examined per locus for a reliable profile. This number is based on 2 parental genomes per individual, or 100 total genomes. Although the electrophoretic separation of gene products is a sensitive tool for examining population structure, it does not detect all changes at the gene level (Nei 1975). So the true amount of variation is underestimated; this fact should be carefully considered, especially in monitoring populations as they become adapted to the laboratory.

Decay of Variability

Random events—genetic drift and founder effect

If unopposed by directed events like selection, random processes may reduce variability by eliminating some alleles. Loss in diversity of genetic material can be caused by both random and directed processes. Decay actually begins at the time wild material is selected for propagation in the laboratory (founder effect). "Bottleneck effect" describes differences in genotypic variability that occur after wild insects have been incorporated into a rearing program—with a decline in variability comes an associated loss of adaptive potential. But much of the heterozygosity present in wild insects may be nonadaptive. So, even if gene frequencies are squeezed at the time of colony establishment, the bottleneck effect may not be severe.

Because of the founder effect, which is a form of sampling error, only part of the wild gene pool is actually incorporated into the laboratory stock. The founder effect

is an extreme example of genetic drift (Wright 1977), which is the change in gene frequencies in a population due to random events. Loss of genes during establishment of the laboratory stock restricts the adaptability of the colonized material. For many species, counteracting this source of decay may require that new genes be added early in a mass-rearing program. At least, a profile of the wild material from throughout its range should be compared to a profile of the founder stock at the time of colony establishment. Either external or internal markers would be useful for this comparison.

Genetic drift is the most important of the random processes influencing gene frequencies in a laboratory colony of insects. Even mutation rates of 10^{-5} – 10^{-6} do not change genetic content of a population as significantly over the short term. In larger populations, the decay due to drift is less extreme than in smaller ones.

As population size varies after colony establishment, more genetic decay occurs, especially if the number of insects declines. Loss due to disease or arbitrary culling, for example, will reduce the effective population size (N_e). Also, to make rearing on a large scale more manageable, colonies are usually broken up into subpopulations that may actually undergo varying degrees of drift. One cage of adults, for example, may have a distortion in sex ratio, with females far outnumbering males. In such a case, the N_e -value would diminish because males would be inseminating more females.

Directional events

Inbreeding.—Inbreeding generally causes genetic decay, as heterozygous genotypes are lost and homozygosity increases. During inbreeding, many of the recessive alleles that were not being expressed in the heterozygous condition can be uncovered. If harmful, these recessives in the homozygous condition may cause inbreeding depression or reduced fitness. The simplest case to consider is a diploid population where a particular locus has two alleles—a dominant wild type, A , and a recessive mutant, a . Matings between two heterozygous individuals, Aa , will result in 50% of the progeny being heterozygous. With random mating among progeny genotypes and an infinite population size, the proportion of heterozygous individuals in the next generation would again be 50%. But no population, laboratory or natural, breeds completely at random or has infinite size. So AA genotypes inbreed, as do aa genotypes. This inbreeding causes decay because the number of heterozygous individuals declines. Through hybrid vigor, heterozygous individuals may be better adapted than either homozygous genotype. Neither overall gene frequencies nor amounts of variation change directly because of inbreeding, but the proportions of genotypes do.

When closely related individuals mate among themselves, homozygosity increases across all loci. This is the primary effect of inbreeding. If phenotypes controlled by one gene mate assortively, then that locus only (and sometimes associated loci) will undergo homozygosis. Such single-gene effects may not seriously influence the amount of heterogeneity. But, in a laboratory colony, they could further the process of subdivision in one subpopulation.

Not all colonies undergo inbreeding depression. For example, I made allozyme comparisons among four wild populations of the mosquito *Anopheles albimanus* (Wiedemann) and a stock that had been maintained at the U.S. Agricultural Research Service's Insects Affecting Man and Animals Research Laboratory in Gainesville, Fla. I found that the colonized strain lost little of its variability, even after 40 generations (table 1). In these studies, I used H and P values for several loci whose mode of inheritance had been determined. Similar comparisons by Bartlett (1980) of the pink bollworm, *Pectinophora gossypiella* (Saunders), also revealed little change in the genetic content of a laboratory strain. Another electrophoretic study, of 12 loci, was conducted by Pashley and Proverbs (1981) on the codling moth, *Laspeyresia pomonella* (Linnaeus). Although they noted a slight loss, their findings showed that heterozygosity values did not change significantly over 25 generations. The adaptive value of the structural loci examined electrophoretically in these studies was not known. But none of the three colonies declined in variability. And the similarity of their genetic profiles suggests that genetic compatibility can be maintained between longstanding laboratory stocks and wild insects.

Wahlund's effect.—A laboratory colony that is divided into several isolates, as is often done in mass-rearing programs, may decline in genetic diversity because it loses heterozygous genotypes. This decline, known as Wahlund's effect, or the stratification principle, occurs if gene frequencies differ among the isolates. The result is an average simulated inbreeding effect across all isolates despite random mating in any one subpopulation. Homozygous genotypes increase and heterozygous ones decrease as a function of the variance in alleles between isolates. This variance is generally defined as the difference between the observed frequency of the average homozygote, aa , among isolates and the expected homozygote frequency obtained by squaring the average of the frequency of the recessive allele of all subpopulations.

In insect colonization, Wahlund's effect is important as a potential source of genetic decay. If the mating pattern of the insect is known, subpopulation structuring and its associated simulated inbreeding can be distinguished from true inbreeding as a contributor to decay, and ap-

Table 1.—Gene frequencies in populations of *Anopheles albimanus* (Wiedemann)

Locus	Frequencies in—				
	Natural populations in El Salvador				A laboratory ¹ population— SANTA TECLA
	1 (\bar{H} =0.21; P =0.59)	2 (\bar{H} =0.28; P =0.67)	3 (\bar{H} =0.25; P =0.61)	4 (\bar{H} =0.14; P =0.51)	
$PGM_1^{1.00}$	0.86	0.81	0.84	0.72	0.87
$PGM_1^{0.95}$.14	.19	.16	.28	.13
$PGM_2^{1.00}$.99	.96	.99	.91	.99
$PGM_2^{1.02}$.01	.04	.01	.09	.01
$IDH_1^{1.00}$.92	.86	.89	.90	.96
$IDH_1^{0.91}$.08	.14	.11	.10	.04
$ALDOX_2^{1.00}$.98	.99	.99	.99	.99
$ALDOX_2^{0.94}$.02	.01	.01	.01	.01
$HK_1^{1.00}$.74	.82	.77	.83	.88
$HK_1^{0.98}$.26	.18	.23	.17	.12
$HK_4^{1.00}$.83	.83	.88	.91	.93
$HK_4^{0.96}$.17	.17	.12	.09	.07

¹Laboratory colony maintained at the U.S. Agricultural Research Service's Insects Affecting Man and Animals Research Laboratory, Gainesville, Fla.

\bar{H} =Population heterozygosity. P =Percentage of polymorphic loci.

appropriate measures can then be taken to offset the decline. Hartl (1980) took another view of Wahlund's effect. He suggested that it can be, on a limited scale, one means of negating subpopulation effects. Hartl refers to the "isolate-breaking" character of Wahlund's effect, where isolated subpopulations behave genetically as though fused together so that heterozygosity levels increase. In natural populations, such fusion, which leads to isolate breaking, is done through migration; however, in the laboratory, this could be simulated through controlled mingling of isolates before release.

Directional selection.—Selection operates continuously during the establishment and maintenance of a colony. The all-too-familiar problems that accompany the early generations are exasperating evidence of the action of directional selection, one form of which is artificial selection. Usually, the traits being selected are quantitative or continuous; so several genes may be contributing to the final phenotype. These polygenic traits that a new colony must contend with include fecundity; developmental rates; longevity; sex ratio; dietary range; photoperiod; and preferences in temperature, humidity, and host. If the uniform environment of the rearing facility is to accommodate all these environmentally sensitive

genes, then directional selection must differentiate each appropriate genotype to mold the insect to the rearing conditions. In the early stages of establishment, directional selection filters out insects that cannot adjust to the extensive change. This action contributes to genetic decay and accounts for the high mortality of newly introduced material. It may also be responsible, in part, for some of the mysterious reductions in size that afflict mass-reared colonies from time to time. After colony establishment, the maintenance procedure automatically maximizes production by selecting as parents the most fit individuals. Here, directional selection is artificial selection in the truest sense, because any phenotype may be refined to any degree by the rearing team. Such a concerted selection regime, involving quantitative traits, is called truncation selection (Wright 1977). In the mass-reared colony, such traits as fecundity, dietary preference, and longevity should be the most susceptible to truncation selection. In any population, the variation of any one of these three traits follows a normal distribution curve. Depending on the needs of the control program, a cutoff, or truncation, point is chosen for a phenotype. This truncation point determines the precise portion of each generation's progeny that will be parents for the next generation. Individuals with phenotypes above the

truncation point are saved while all others are discarded. The truncation point lies closer to the mean phenotype of the most fit individuals for the character being selected than it does to the mean phenotype of the whole population.

Through directional selection, genotypes adapt to unvarying laboratory conditions and so lose their ability to survive in varying environments. While this loss helps efficient production, the insect produced is highly inflexible and could not be expected to compete with its wild counterparts. To neutralize the effects of truncation selection, environmental conditions should be varied in the insect-rearing facility.

Methods of Maintaining Heterogeneity in a Laboratory Colony

Modes of selection

The means that sustain genetic variability in natural populations can be used as models for ways to maintain it in the laboratory. Sources of decay in the laboratory can be offset by several different types of selection and by other phenomena. One type of selection, disruptive or centrifugal, can be multidirectional either across individuals in one local population (deme) or across demes in a species (Wright 1977). The net effect is a mosaic distribution of allelic frequencies in a spatially restricted breeding group. Density-dependent (Clarke 1972) and frequency-dependent (Gromko 1977) selections promote changes in the frequencies of opposing alleles. Given alternate conditions, the number of such alleles will either increase or decrease so that they always have inverse frequencies. Group selection favors the survival of the population over that of the individual (Hartl 1980). In gametic selection, an allele that would be lost in a gamete may be maintained in a zygote. Overdominance, or heterozygote superiority, is another means of maintaining alleles in a population (Dobzhansky 1970); and, in the hitchhiking effect, a neutral allele may fluctuate because it is physically associated with a selected locus (Thompson 1977).

Precolonization methods— pooled multiple-founder colonies

Precolonization and postcolonization events affect the establishment and maintenance of variability. Partial establishment of heterogeneity, for example, can occur when insects are field-collected for propagation. In nature, species consist of genetically unified, yet heterogeneous, populations that are adapted to the local environment. If insects intended for establishing a laboratory strain come from one population, the adaptability of the colony is limited; and release insects may not interact effectively

or uniformly throughout the range of the target pest. Selection and pooling of founder insects from throughout the range of the species can provide a much wider representation of the gene pool. The wider representation will insure that laboratory material has greater fitness. Initially, insects from a variety of geographical areas should be mingled in the laboratory. The insects must, of course, have reproductive compatibility. Although reproductive isolation is the basis of speciation (Mayr 1963), its occurrence in *C. hominivorax*, a major pest species, added a surprising consideration to control programs. Makela and Richardson (1978) reported that hidden reproductive isolates were present in the range of *C. hominivorax*; these isolates contributed to mating incompatibility between the release and field populations. The use of pooled multiple-founder stocks or colonies to establish a laboratory strain would preclude this source of incompatibility.

Postcolonization methods

Variable laboratory environment.—For a laboratory colony of insects, even minor changes in rearing conditions can affect variability levels. A static environment leads to a static genotype and ultimately to less fit insects. Environments that are varied over space (Hedrick et al. 1976) and varied over time can contribute enormously to the flexibility of an ongoing colony; the adaptive challenges will be continual. Certainly, the dynamic environment of the target populations is more closely simulated by such a variable laboratory environment. The concept is simple; putting it into practice in a large-scale rearing program is not. One approach would be to pool insects from parallel but environmentally different subcolonies just before release. The number of different subcolonies required would depend on the biology of the insect and the type of control program, but procedures could be varied in the same rearing facility. Some variables to consider with this approach are population densities and sex ratios; temperature, humidity, and dietary preferences; and container sizes. Heterogeneity could also be maintained by varying the regime of each subcolony by, for example, rotating parallel subcolonies through the environments that were available in the facility. Such a program is feasible in most of today's mass-rearing facilities.

Gene infusion.—Gene transfer between wild populations usually occurs through migration. In reared insects, the analogous process is gene infusion. New genes can be obtained either from natural populations or from parallel subcolonies. Alleles introduced in this way have the same limitations as a new mutant, particularly if a rare gene is involved. During the life of a colony, the gene pool should be rejuvenated occasionally with wild insects. Introducing wild stock to the established colony restores some of the heterogeneity that becomes lost because of drift or inbreeding. The infused genes also help to maintain genetic

similarity between field and laboratory material; and they augment mutation as a source of new, or unique, alleles in the colony. Finally, through selective monitoring of both field and laboratory populations (with electrophoresis, for example), specific genes can be selected from the donors to help genetically tailor the recipients. Because the laboratory colony is a subpopulation of the species it belongs to, gene infusion cements its genetic relationship to the wild populations. So gene infusion offsets the isolate-forming tendency of Wahlund's effect.

Effective population size.—Many reductions in colony size that are caused by directional selection can be traced to a specific environmental factor. Often the agent is a subtle one, such as a change in a manufacturer's dietary ingredient or inadvertent use of cage materials that have been treated with a toxin. These reductions decrease the effective population size (N_e) of the colony and must be compensated for by adding outside individuals. To maintain sufficient heterogeneity, a colony should not decline below the number of founder insects. In natural populations, several factors influence the N_e value. In *Drosophila* species, such agents as disease, predation, competition, or bad weather may require an effective population size of 1,000 or more during warm weather, but only about 100 when densities decline in winter (Begon 1977). Laboratory colonies do not experience predation or hostile climatic conditions but are subject to disease and competition. These can reduce the N_e of the laboratory colony to about 500. For mass-rearing programs, this figure refers to the number of adults in a subpopulation cage. The maintenance of a high N_e reduces the effects of random drift and inbreeding and helps to offset decay.

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Section 2

Diets and Containerization for Insect Rearing

The evolution of artificial diets, assurance of diet-ingredient quality, and new container designs have had a major impact on the development of basic and applied entomology. The rearing of insects on artificial diets has been confined to this century, especially to the last two to three decades. (The published research involving laboratory-reared insects amounts to about 50% of recent entomological publications.) Artificial diets relieve researchers from the trouble and expense of maintaining greenhouse facilities and host plants for rearing phytophagous insects. Continual advancements in such diets and improvements in containerization have made it possible for many additional species of insects to be reared. Also, new in-field strategies for controlling pest insects—the sterile-male technique, augmentative release of parasites and predators reared in vivo and in vitro, and application of microbial pathogens produced directly or indirectly on artificial media—have placed new emphasis and dependence on insect rearing.

To have a consistent, economical production of vigorous, competitive insects (phytophagous or entomophagous), a greater understanding about feeding is needed for each phase of the development cycle in relation to behavior, preference, nutritional requirements, etc. The diet should insure proper insect nutrition at the lowest possible cost and yet be practical to use. So, further research is required for improving existing diets and formulating new diets for those species that cannot be reared at the present time.

Since mass production has become more common, future advances will depend on several things, including standardizing diets and developing quality-control standards

for the diets and their individual components. Many commercial dietary ingredients are presently subjected to standardized analytical tests such as those found in the Official Methods of Analysis of the Association of Official Analytical Chemists. But, little or no technical data are provided for some commercially available products used in artificial diets. In the final analysis, bioassays are probably more important than chemical assays in determining an ingredient's nutritional value. A bioassay answers the question "what is the ultimate effect of the diet on the reared insect?" The size and importance of the rearing operation should determine the resources that can be used for dietary assays. The handling and storage of incoming ingredients are also important to overall diet quality.

No discussion of artificial diets for insects would be complete without giving consideration to rearing containers and their closures. Many different kinds of containers have been used that were adapted from currently marketed items. As artificial diets were improved and more were used, certain containers became generally accepted because they provided favorable microenvironments for the diets and insects. Sizes and shapes of containers vary according to the type of diet and the behavior of the insect. Some desirable characteristics shared by most insect-rearing containers and closures include the prevention of microbial contaminants and pathogens, allowance for proper gas exchange, prevention of escape, moisture regulation, economics, visibility and accessibility, convenience of handling and harvesting, and ease of cleaning and disinfection. Additional research in containerization might well center on design improvements for the particular species and stress the use of reusable containers.

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Insect Diets

Historical Developments, Recent Advances, and Future Prospects

By Pritam Singh¹

Introduction

The continuous availability of large numbers of uniform laboratory-reared insects of acceptable quality has been an important contribution to the development of modern experimental and economic entomology. Successful rearing of these insects has depended on sound knowledge of insect biology, behavior, habitat, and nutrition. An understanding of the mating habits, preoviposition and oviposition periods, fecundity, longevity, sex ratio, environmental requirements, and food and feeding preferences of the insect is necessary in developing rearing techniques. So insect rearing is a complex field closely related to other disciplines, especially dietetics and nutrition. Here, "dietetics" is the study of the kind and quantity of food that will be eaten. "Nutrition" refers to the specific, chemically defined components that the insect must have to grow, reproduce, and perform as it should. This paper reviews the development of insect dietetics and discoveries about insect nutrition. It also discusses recent advances in insect diets and future needs and possibilities. Finally, it lists the most important references on various aspects of insect diets.

Historical Developments in Insect Diets

Milestones in the development of insect diets

General insect rearing.—The pioneering work on artificial diets for insect rearing was done by Russian and French scientists in the early 1900's. Bogdanow (1908) was the first to rear an insect axenically from egg to adult when he reared the blow fly, *Calliphora vomitoria* Linnaeus, on a diet compounded from peptone, meat extract, starch, and minerals. Later, Loeb (1915) succeeded in rearing *Drosophila* spp. for five generations on a diet of grape sugar, cane sugar, ammonium tartrate, citric acid, dipotassium hydrogen phosphate, magnesium sulfate, and water. Guyenot (1917) was the first to rear an insect (*Dro-*

sophila ampelophila Loew) on a completely artificial diet. And the first multicellular organism to be reared axenically from egg to adult on a chemically defined diet was *Drosophila melanogaster* Meigen, which Schultz et al. (1946) reared on pure amino acids, minerals, vitamins, etc. Hawkes (1920) was partly successful in feeding the larvae of the twospotted lady beetle, *Adalia bipunctata* (Linnaeus), a coccinellid, on cooked or raw chicken eggs and powdered dates. And Szumkowski (1952) was the first to rear the predatory lady beetle, *Coleomegilla maculata* DeGeer, on mammalian liver enriched with vitamin C. Later, Szumkowski (1961) developed a mixture of fresh yeast with glucose or sucrose solution that was better and thus replaced the liver diet. Atallah and Newsom (1966) improved on Szumkowski's diet by formulating an artificial diet that fed eight successive generations of *C. maculata* and allowed the females to oviposit into diet encapsulated in a sealed Parafilm tube where the eggs hatched and the larvae developed to maturity.

Zabinski (1926, 1928) reared the oriental cockroach, *Blattella (=Periplaneta) orientalis* Linnaeus, and the German cockroach, *Blattella germanica* (Linnaeus), on 18 parts of ovalbumin, 56 parts of starch, 20 parts of saccharose, 2.3 parts of agar, and 3.7 parts of a salt mixture. Later, House (1949) formulated a chemically defined diet for *B. germanica*. Several stored-product pests were reared on a casein-based diet by Fraenkel (1943) and his associates in the early 1940's. These achievements have been reported in earlier reviews, first by Uvarov (1928) who listed 600 titles, then by Trager (1941, 1947). Singh (1955) was the first to report a chemically defined diet for the yellow-fever mosquito, *Aedes aegypti* (Linnaeus); this diet has formed the basis for rearing other species of mosquitoes for nutritional studies. Rearing of hemipterous insects on diet was not achieved until the mid-1950's when Scheel et al. (1957) succeeded with the large milkweed bug, *Onco-peltus fasciatus* (Dallas), and the onespotted stink bug, *Euschistus variolarius* (Palisot de Beauvois). Aphids apparently were more difficult than other insects to culture and were first reared by two independent groups: In the United States, Mittler and Dadd (1962) reared the green peach aphid, *Myzus persicae* (Sulzer); in Canada, Auclair and Cartier (1963) reared the pea aphid, *Acyrtosiphon pisum* (Harris). Since then, over 30 species of aphids have been successfully reared on chemically defined diets, some for several generations (Kunkel 1977).

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Rearing phytophagous insects.—Bottger (1942), working in South Africa, was the first to report the use of an agar-based diet for rearing of a phytophagous insect, the European corn borer, *Ostrinia nubilalis* (Hübner). The diet was compounded from casein, sugar, fats, salts, vitamins, cellulose, agar, and water. This work was followed by that of Beck et al. (1949) at the University of Wisconsin, whose diet for *O. nubilalis* was formulated from highly purified natural products containing an unidentified growth factor that was later identified as ascorbic acid by Chippendale and Beck (1964). In Japan, by including in the diet an extract of the host plant used in the *O. nubilalis* diet developed by Beck et al. (1949), Ishii (1952) successfully reared the Asiatic rice borer, *Chilo suppressalis* (Walker), and Matsumoto (1954) successfully reared the oriental fruit moth, *Grapholitha molesta* (Busck). Vanderzant and Reiser (1956) used an aseptic technique to rear the pink bollworm, *Pectinophora gossypiella* (Saunders), on a diet devoid of plant extracts. Since then, several phytophagous insects have been reared on diets compounded from pure chemicals and other nutritive substances completely unfamiliar to insects.

One of the most important advances in rearing lepidopterous and other phytophagous insects was the use of wheat germ as a primary nutrient source in formulating insect diets. This work was done at College Station, Tex., by Adkisson et al. (1960) for the pink bollworm. Berger (1963) further developed the use of this wheat-germ-based diet for the large-scale rearing of *Heliothis* spp. These two diets, with modifications, have been the basis for rearing many other insects (for example, see Ignoffo 1963). Since then, wheat germ has been used in several hundred insect diets (see listings in House et al. 1971 and Singh 1974b, 1977a, 1977b). Shorey and Hale (1965) used beans as the primary protein source in a diet to rear nine species of noctuid larvae. Several varieties of beans have since been used for similar purposes in the formulation of lepidopterous diets.

Rearing entomophagous insects.—Yazgan and House (1970) achieved a breakthrough in rearing an ichneumonid endoparasitoid, *Itopectis conquisitor* (Say), on a chemically defined diet in aseptic conditions; and Yazgan (1972) obtained 62% fecund adults from neonate larvae reared on this diet. But House (1978) was the first to achieve complete success by using an encapsulated medium for rearing this species from egg to adult. Also, Hoffman and Ignoffo (1974), using a semisynthetic medium, reared an endoparasitoid wasp, *Pteromalus puparum* (Linnaeus), with 44% adult emergence from neonate larvae. Thompson (1975) succeeded in rearing an ichneumonid ectoparasitoid, *Exeristes roborator* (Fabricius) on artificial diet, with 80% survival. Hoffman et al. (1975), using a synthetic diet, were the first to rear

an egg parasitoid, *Trichogramma pretiosum* (Riley), from egg to adult in vitro. Several studies have concentrated on developing an artificial diet for rearing the common green lacewing, *Chrysopa carnea* Stephens. Hagen and Tassan (1965, 1970), for example, succeeded with an adult diet containing enzymatic protein hydrolyzate and Wheast, while Ridgway et al. (1970), also using artificial diet, described a mass-rearing technique for *C. carnea*. Vanderzant (1969b) reported the use of a semidefined diet for rearing *C. carnea* larvae, and Vanderzant (1973) discussed improvements in the diet. Martin et al. (1978) mechanically encapsulated Vanderzant's diet in paraffin wax, candelilla wax, polyethylene, and polybutene for purposes of mass rearing *C. carnea*; the capsule is easily consumed by second- and third-stage larvae, but first-stage larvae have difficulty penetrating it. Mass-production techniques using artificial diet for rearing *C. carnea* and other entomophagous arthropods have been reviewed by Morrison and King (1977). Currently, the technology for rearing parasites and predators on artificial diets is far less developed than the technology for rearing Lepidoptera, Coleoptera, and Diptera.

Factory or mass rearing on artificial diets.—Several insects have been mass-reared successfully on different artificial diets and used for various insect-control programs. Some examples are: European corn borer for virus and plant-resistance studies; tobacco hornworm, *Manduca sexta* (Linnaeus), for hormone studies; nutsedge moth, *Bactra verutana* Zeller, for control of purple nutsedge; saltmarsh caterpillar, *Estigmene acrea* (Drury), for virus production; gypsy moth, *Lymantria dispar* (Linnaeus), for virus production; and several species for programs using sterile-male release, including *Culex fatigans* Wiedemann; horn fly, *Haematobia irritans* (Linnaeus), and stable fly, *Stomoxys calcitrans* (Linnaeus). Recent developments in the mass production of tsetse fly, *Glossina morsitans* Westwood, feeding on citrated blood via membranes have made large-scale colonization possible (Mews et al. 1977). The commercial production of crickets, blow fly larvae, and *Tenebrio* larvae as baits for the fishing industry is well established in Europe and the United States.

The rearing of the screwworm, *Cochliomyia hominivorax* (Coquerel), by Melvin and Bushland (1936) on a mixture of milk, blood, lean beef, and formaldehyde is another milestone in insect rearing, as these were the first blood-feeding insects to be reared on an artificial diet. In 1978, 10 billion flies were produced for release in Mexico and southern Texas as part of a sterile-male release program (Bush 1978). The success of this effort has led to other, similar programs also based on mass-rearing techniques developed earlier. For example, in the mid-1960's the boll weevil, *Anthonomus grandis grandis* Boheman, was mass-produced on artificial diet by Gast and Davich (1966), and the R. T. Gast Rearing Laboratory at

Mississippi State, Miss., has since produced as many as 3–6 million boll weevil adults per week (Griffin et al. 1979). Martin (1966) and Mangum et al. (1969) described a mass-rearing procedure using artificial diet for the pink bollworm that enables production of more than 1 million moths per day in the U.S. Animal and Plant Health Inspection Service's rearing facility at Phoenix, Ariz. Hamilton and Hathaway (1966) developed a mass-production method using artificial diet for the codling moth, *Laspeyresia pomonella* (Linnaeus), that is presently used at the U.S. Agriculture Research Service's (ARS) laboratory in Yakima, Wash. This facility can now produce 3 million moths per year; using similar procedures, the Canadian Department of Agriculture's facility in Summerland, British Columbia, can produce 2 million moths per month (La Chance 1974). A technique that includes a prototype machine for dispensing artificial diet for the corn earworm, *Heliothis zea* (Boddie), was developed at the ARS Southern Grain Insects Research Laboratory in Tifton, Ga., where up to 1 million eggs/day could be produced (Burton 1969). The increase in production of the corn earworm from a few hundred per day to about 120,000/day was achieved mostly through this and similar mechanization; from March 1972 to February 1974, 6 million pupae were produced (Sparks and Harrell 1976). Researchers at the ARS Cotton Insect Research Laboratory in Brownsville, Tex., developed a technique and a low-cost soyflour and wheat germ diet for mass rearing the tobacco budworm, *Heliothis virescens* (Fabricius); production was stabilized at about 70,000 pupae/day, and pupae were shipped to St. Croix, U.S. Virgin Islands, for moth emergence and release as part of a test of sterile-male release (Raulston and Lingren 1972). The mass-production method described by Henneberry and Kishaba (1966) for rearing the cabbage looper, *Trichoplusia ni* (Hübner), on artificial diet was used to produce 10,000 adults/week at an average cost of \$0.36/pupa. Recently, Chauthani et al. (1971), Poitout and Bues (1972), and Vail et al. (1973) gave improved formulations for diets to rear cabbage loopers for production of *Bacillus thuringiensis* Berliner and viruses.

The house fly, *Musca domestica* Linnaeus, and the little house fly, *Fannia canicularis* (Linnaeus), were first successfully colonized by Lodge (1918) in England. He used a mixture of casein, bread, water, and banana surrounded by a layer of dry rubbish where the maggots could pupate. In the United States, Glaser (1927) continuously reared the house fly on horse manure plus yeast. This diet, with slight modifications, was the basis of most house fly rearing until Richardson (1932) introduced CSMA (for Chemical Specialties Manufacturers Association), a diet that provides year-round rearing on an efficient medium. It is now possible to mass-produce millions of house flies on diet, as is currently being done at the ARS Insects Affecting Man and Animals Research

Laboratory in Gainesville, Fla., where 6 million flies are produced each week for the production of 4–5 million *Spalangia endius* parasites. The house fly has also been mass-produced on diet for sterile-insect programs in Italy and the Bahama Islands.

Recent Advances in Insect Diets

In recent years, much has been learned about insect dietetics and nutrition and about ways to protect insect diets from chemical and biological, especially microbiological, contamination. The information that follows summarizes these recent advances.

Dietetics

Over the past 20 years or so, much effort has been expended combining the 40 to 50 nutrients common to most foodstuffs into acceptable food for insects. Researchers have learned that, to encourage consistency in compounding, the raw materials should be generally available, economical, uniform in nutrient density, and of unvarying quality. Adequate chemical analyses must be conducted to aid the selection and formulation of diet ingredients and to insure freedom from degradation, infestation, contamination, or other harmful changes. Texture, shape, particle size, and other physical characteristics of the diet are also important, depending on the feeding habits of the insect.

Nutrition

The choice of a specific food by a species is often determined by nonnutritional factors such as physical properties and phagostimulants. So proteins, carbohydrates, lipids, and vitamins must all be present in adequate supply since not having enough of one nutrient can cause the insect to use the food more slowly and less efficiently than it should. An unsatisfactory nutrient balance may lead to nutritional diseases affecting growth, development, reproduction, and other life processes (Friend 1959, House 1959, 1961b, 1963, 1965). So House (1966) proposed three principles applicable to insect nutrition: (1) The rule of sameness—all insects need the same nutritional quality whatever their feeding habits and systematic position of classification, (2) the principle of nutrient proportionality—normal nutrition requires that nutrient proportions are metabolically suitable (there may be several equally good nutrient balances), and (3) the principle of cooperating supplements—supplementary sources of nutrients may be important to most insects.

Sources of protein and energy are the largest parts of a diet. The protein content of the diet should ideally contain all 10 essential amino acids in the correct proportions and be readily digestible so that they are available

to the insect. The amount of protein required in a diet is influenced by its nutritional quality, which is determined not only by its amino acid composition, but also by how efficiently the digested food is used (Vanderzant 1973). Nutritional quality may be seriously impaired by treatment during processing, particularly by overheating, which can destroy amino acids and cause indigestible or otherwise unavailable complexes to form. One of the two most vulnerable and easily measured constituents is lysine, and it is generally used as an indicator of protein quality. The most common protein sources used in insect diets are casein, egg albumen, lactalbumin, and soy protein.

When an insect is allowed unlimited access to food, it will eat at least enough to satisfy its energy requirements. Carbohydrates (starches and sugars) are the major energy source in most diets. Sugars also serve as phagostimulants. Fats give more than twice the energy of sugars or starches. Proteins also supply energy, but are an expensive source. So insects are likely to eat less of a high-energy diet than they would of a low-energy one. Then the intake of the other ingredients will be proportionately less, and their concentration must be increased so that the amounts ingested do not fall below what is needed.

The dietary requirements for lipids, fatty acids, and sterols in insects have been thoroughly documented (see Clayton 1964; Fast 1964, 1966; and Gilbert 1967). They provide energy; they are also essential to the development of wing buds—deficiencies, especially of linoleic and linolenic acids, cause deformed wings in adult Lepidoptera (Chippendale et al. 1965). Unlike mammals, insects cannot synthesize the steroid ring. So cholesterol, the most common sterol in insects, must either be obtained from dietary sources or be added in pure form. Cholesterol is a precursor of ecdysone, the molting hormone. Insect sterol nutrition and metabolism are reviewed by Clayton (1964) and Robbins et al. (1971).

Vitamin C (ascorbic acid) is in the diet for most phytophagous species, though stored-product insects, flies, and cockroaches can grow without it. Fat soluble vitamins such as A, D, E, and K are often unnecessary. But vitamin A is required by some insects for normal vision, and vitamin E has been associated with reproduction. The vitamin B complex—including thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, folic acid, and biotin is required, though cobalamin (B_{12}) is not clearly necessary to insects. Mineral requirements are more difficult to assess because excluding particular inorganic ions from synthetic ingredients grossly alters the balance of those remaining. Nevertheless, sodium, potassium, magnesium, chloride phosphates, and minor elements in-

cluding iron, copper, zinc, and manganese are necessary for optimal growth.

A major difficulty in compounding diets for chewing insects is to provide solidity with a water content of 80% or more. Agar is most commonly used because it forms a rigid gel at low concentrations of 2%–3% in most diets. But agar is expensive, and replacements must be sought for use in mass rearing. Agar may be reduced by increasing the fiber content. Fiber contributes little to overall nutrition but is essential for binding the nutrients, providing bulk, and giving proper dietary shape and texture.

Microbial contamination

Generally, micro-organisms in artificial diets cause spoilage (Clark et al. 1961, Ludemann et al. 1979), alter the biological performance of the insect (Singh and House 1970a, 1970b, 1970c; Singh and Bucher 1971), and may harm symbionts in the gut (Buchner 1953; Fraenkel 1959a, 1959b; Brooks 1960, 1964; N.C. Pant 1973b). The microbial contaminants most often encountered in artificial diets are *Aspergillus* yeasts, *Rhizopus* bacteria, and *Penicillium* molds. Several species of these organisms may often be found on one diet. The antimicrobials commonly used to combat these organisms include formaldehyde, methyl *p*-hydroxybenzoate, sodium benzoate, potassium sorbate, sorbic acid, streptomycin, penicillin, and Aureomycin (chlortetracycline).

In reviewing what is known about how antimicrobials affect insects, Singh and House (1970b) examined what is called the safe level. A compound's safe level is the concentration that does not reduce the yield of pupae and adults or increase the time for larval development by more than 25% of normal. Above the safe level, antimicrobials are harmful in proportion to the concentration used (Singh and House 1970b, Singh and Bucher 1971); insect size may be reduced, larval life prolonged, and mortality in larval and pupal stages increased (Singh and House 1970a, 1970c). The ideal antimicrobial food additive should suppress a wide variety of micro-organisms at a concentration safe for the insect.

If possible, diets should be sterile, and this can be achieved by autoclaving at 15 lb/in² of pressure for 15–20 minutes or by other means such as irradiation, chemical, flash, or gas sterilization. Microbial growth can also be prevented in diets by including mold inhibitors and antibiotics or by adjusting the pH. Such procedures are satisfactory if the environment is clean, equipment is sterilized, dietary ingredients are not initially contaminated, and eggs are washed in detergent and sterilizing solutions. Rearing laboratories and diets should be monitored regularly for microbial contaminants, and strict sanitation and hygiene standards should be maintained.

Future Prospects for Insect Diets

The developments in artificial diets discussed above have made both small- and large-scale insect rearing possible. But many problems remain, and researchers are concentrating on resolving these. More must be learned about how diet ingredients affect insect quality. More must be learned about the dietary needs of host insects in production of parasites and pathogens. Optimum diets must be developed for species that are not yet reared on artificial diets. And ways to standardize diets must be explored.

Quality control

The performance of laboratory-reared insects is affected by many factors that must be controlled for production of a uniform, high-quality insect (Boller 1972; Mackauer 1972, 1976; Hoy 1976; Huettel 1976, 1977; Boller and Chambers 1977a, 1977b). Chambers (1977) listed the critical performance traits as vigor, irritability, activity, sound production, response thresholds, reproductive potential and drive, biotic potential, and others. Changes may occur in metabolic functions such as CO₂ output and nutritional needs; in tolerance to temperature, irradiation, or other physical factors; in fertility, fecundity, longevity, or population-stress tolerance; and in biological conformities such as rhythms, mating behavior, host specificity, other chemical and physical responses, pheromone production, and mate recognition. Much research still must be done on how diet ingredients affect insect quality, so that quality-control procedures in mass-production programs can be suitably adjusted. Some preliminary work has already been done on how diet affects pheromone production, enzymes, and vision.

Dietary effects on pheromone production.—Pheromone precursors may be needed in the diet of insects mass-reared for use in sex-pheromone traps used to monitor insect populations. Diets that do not contain natural host material may cause the reared insect to produce insufficient pheromones. For example, laboratory-reared boll weevil males are as attractive as natives if they have access to cotton squares and flowerbuds as food; but pheromone production is reduced by 50% at 1 hour after such food is removed and by 90% at 24 hours (Hardee 1971). Similarly, the pheromone production of bark beetles is enhanced if they are fed glucose-supplemented diets (Pitman et al. 1966).

Field-collected larvae of the brownheaded leafroller, *Ctenopseustis obliquana* (Walker), can be satisfactorily reared to the adult stage on a general-purpose artificial diet (Singh 1974a). But the species could not be continuously reared on artificial diet in the laboratory because males and females would not mate. Replacement

of 20% of the diet with lyophilized plant powder, from *Acmena smithii* (Poivet), increases pheromone production and therefore mating (R. A. Galbreath, unpublished data). On the other hand, the light brown apple moth, *Epiphyas postvittana* (Walker), has been continuously reared for over 80 generations on the same diet devoid of any plant material. Likewise, Miller et al. (1976) reported that there is no difference in pheromone production between moths of the female oak leafroller, *Archips semifervans* (Walker), reared on artificial diet and those reared on three species of oak (*Quercus* spp.). Also, males fed these diets respond identically in laboratory bioassays and in field tests. These findings conflict with the hypothesis that the composition of moth sex pheromones varies with slight changes in diet (Hendry et al. 1975, Hendry 1976). This hypothesis has been further refuted by Hindenlang and Wichmann (1977) who were among its original proponents. Clearly, more study is needed of the relationship between specific diet ingredients and pheromone production.

Dietary effects on enzymes.—Another concern for researchers is how artificial diets influence insect enzymes. For example, Ahmad and Forgash (1978) reared gypsy moth larvae on a wheat-germ-based artificial diet and on oak leaves and reported that growth, development, and maturation are comparable for the two diets but that activity in the mixed-function oxidase enzyme is higher in larvae reared on the artificial diet. In insects, mixed-function oxidases often affect the duration and intensity of insecticide action and thus influence insecticide metabolism, detoxification, and resistance.

Similarly, Bush (1978) discovered significant differences in the genetic makeup of wild screwworm flies, particularly in certain genes controlling enzymes involved in flight activity. One very important enzyme, α -GDH (α -glycerol phosphate dehydrogenase) exists in two different forms. The factory-reared flies have contained mostly α -GDH₁. And G. B. Kitto (unpublished data) has shown that the two forms of α -GDH have optimal activity at different temperatures; α -GDH₂ is less active than α -GDH₁ in the temperature range experienced in nature. The high constant temperature used to speed development in the factory has apparently been exerting a strong selective force favoring α -GDH₂ over α -GDH₁. This finding also suggests that the competitive ability of the fly in nature might decrease as the frequency of α -GDH₂ increases, because factory flies would have to cope with a wide temperature range in nature; those individuals lacking the α -GDH₁ enzyme simply would not be able to fly as well as wild individuals.

Dietary effects on vision.—Not much is known about how diet affects insect vision. Tryptophane, an important amino acid, is metabolized to ommochromes, a group of vital pigments associated with screening in the insect

eye. Kayser (1979) has demonstrated that the tryptophane requirement in *P. brassicae* is very precise; lower amounts result in fewer metabolites essential for normal vision. Vitamin A is another nutrient associated with vision in many insects and is therefore another important constituent of the diet. Agee (1979) reported on an instrument that measures the visual sensitivity of insects and could be used to monitor these differences. So, more research is needed on the relationship of dietary ingredients and insect vision and on means of monitoring changes in this essential attribute.

Host insects in production of parasites and pathogens

Parasite production.—In parasite production, the diet determines how well the host's nutritional needs are met, which in turn may influence the quality of parasites produced on host insects. For example, when the Asiatic rice borer was reared on artificial diet, its parasite, *Apanteles chilonus* Munakata, was adversely affected (Kajita 1973). Likewise, Etienne (1973, 1974) reported that the tachinid *Lixophaga diatraeae* (Townsend) cannot be continuously reared on larvae of an unnatural host—the greater wax moth, *Galleria mellonella* (Linnaeus)—that has been fed beeswax and pollen unless the host diet is supplemented with vitamin E or wheat germ. In this case, problems in rearing *L. diatraeae* on greater wax moth larvae were eliminated by fortifying the diet with a high-protein cereal and the addition of 120 g of wheat germ per kilogram of diet (Morrison and King 1977, King et al. 1979). Morrison and King (1977) concluded from this result that “suitability cannot be determined merely by screening hosts, but host nutrition and other factors must also be considered, and compromises between entomophagous arthropod quality and quantity may have to be made because of cost and the numbers required.” Current and future research should solve similar problems for other hosts and parasites.

Some progress has been made in rearing entomophagous species (Hoffman et al. 1975) on medium without host involvement; but this type of rearing will be more common and probably more economical in the future. This will be a major advance in parasite production for suppression programs.

Pathogen production.—Rearing larvae on artificial diet to produce virus has many advantages over the conventional leaf-feeding method. For example, the diet can be sterilized to avoid contamination by other microbes, the need for growing plant material is eliminated, and measured doses of the virus can be given more conveniently to the larvae. Tanada (1965) and Helms and Raun (1971) have found that nutrition is important in the susceptibility of insects to virus. Shvetsova (1950) reported

that greater wax moth larvae are most susceptible to nuclear polyhedrosis virus when fed wax enriched with nitrogen and carbohydrate. Pimentel and Shapiro (1962) had similar results with a high-nitrogen diet but found that nuclear polyhedrosis virus does not increase when greater wax moth larvae are fed standard diet or extra carbohydrates. The incidence of virus infection can also be increased by a reduction in certain diet ingredients (David et al. 1972). For example, when sucrose is omitted or the content of casein reduced from a semisynthetic diet for *Pieris brassicae* (Linnaeus), the incidence of granulosis virus disease increases (David and Taylor 1977). Similarly, Shapiro et al. (1978), in examining how diet influences production of nuclear polyhedrosis virus from gypsy moth, found that the total virus yield varies. Virus production is more economical from moths reared on a diet with a high concentration of wheat germ. An increase in vitamin concentration improves virus yield slightly, but few differences are caused by change in pH from 4 to 7.

Clearly, pathogen production is influenced by the nutritional status of the host, which in turn depends on diet. As with parasite production, then, formulations of diets specifically for pathogen production should receive considerable attention from researchers in the future.

Development of optimum diets for new species

Analysis of the reference listings in Singh (1977b) shows that fewer than 1,000 insect species have been reared on artificial diets. Only 160 pre-1950 references were found, but there were more than 2,300 references up to 1978, and the list continues to grow. Most species reared on artificial diet are from the orders Lepidoptera, Coleoptera, and Diptera. Though there are 45 species listed under the order Hymenoptera, 32 of these are ants, and 13 of the ant species have been reared for only part of their life cycle. A review of Singh (1972, 1977a, 1977b) shows that only about two dozen or so species have been successfully reared for several generations. Also, diets for predators, parasites, and blood-feeding insects have received much less attention than diets for plant pests. Though tremendous advances have been made in colonizing some species, much work still remains to be done in developing the best artificial diets and rearing techniques for new species as well as those already colonized.

Standardization of insect diets and rearing methods

It is time to standardize diets, at least for commonly used test insects. Standardization of insect diets is important because the biological (for example, life cycle and fecundity) and chemical (for example, pheromone, hormone,

biochemical, and enzyme) characteristics of the reared insect depend partly on nutrition. So quality standards must be applied to the formulation of artificial diets, including the origin and analyses of commercial ingredients. Diets should be prepared by standard methods and the following variables recorded: percentage of protein, carbohydrate, lipid, minerals, and vitamins; osmolarity; pH; texture; moisture content; microflora activity; use of mold inhibitors; and other secondary substances.

Literature Review

In Singh (1977b), I reviewed the literature on artificial diets for insects, mites, and spiders from 1900 to 1976. Diets were listed for more than 750 species collated from nearly 2,000 references. (See also Singh 1972.) Insect diets have been cataloged in recipe books by House (1967), House et al. (1971), Singh (1974b, 1977a, 1977b) and Gomez (1978a). (See also Gomez's 1978b bibliography of artificial diets for Lepidoptera.) Wyniger (1974) listed some artificial diets. Various matters related to diet have been reviewed by Yushima (1962), Vanderzant (1966, 1969a, 1974), Boness (1968, 1969, 1970), McKinley (1971), and Gardiner (1978). Five general insect-rearing books (Ishii 1959; Needham 1959; Smith 1966, 1967; and Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture 1968) have useful discussions of artificial diets.

Insect nutrition has been exhaustively reviewed by House (1961a, 1962, 1965, 1972, 1974), Patton (1963), David (1967), Guennelon (1967), Gordon (1968), Chapman (1969), Dadd (1970, 1973), Hodgson and Rock (1971), N. C. Pant (1973a), and Levinson (1976). Specialized reviews are available on honey bees, *Apis mellifera* (Linnaeus), by Haydak (1970); on the silkworm, *Bombyx mori* (Linnaeus), by Ito (1967, 1972, 1979); on *Drosophila* by Sang (1978); on Diptera by Friend (1968); on phytophagous insects by Friend (1959), McGinnis and Kastings (1964), Beck and Chippendale (1968), and J. C. Pant (1973); on locusts and grasshoppers by Dadd (1963); on aphids by Auclair (1963), Yushima (1968), Massonié (1971), and Kunkel (1977); on muscoid flies by Spiller (1964); on plant-sucking insects by Auclair (1969); on natural enemies by House (1977); and on stored-product pests by Punj and Girish (1968) and Misra (1973). Altman and Dittmer (1968) and Dadd (1977) give comprehensive tabular summaries of quality requirements for insect nutrition. And Rodriguez (1972) presents several papers on the significance and implications of insect nutrition in ecology and pest management.

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Ingredients for Insect Diets

Quality Assurance, Sources, and Storage and Handling

By F. D. Brewer¹ and Oliver Lindig²

Introduction

Analysis of an insect's natural food source often suffices as a basis for formulating an artificial diet for the insect. But an acceptable artificial diet must have more than the right ingredients. The ingredients must be of the quality needed to rear the insect for a particular purpose. Of course, even if the diet is imperfect, it may be satisfactory, and the insects may adapt to it quickly (Davis 1972) whatever the sources of the dietary components (proteins, fats, etc.). This paper discusses ways to assure ingredient quality, sources of dietary components, and general considerations for storage and handling of diet ingredients to maintain quality.

Assuring Quality of Diet Ingredients

Most processors and suppliers of diet ingredients will furnish, on request, fairly detailed technical data on their products, assurances of product quality, and manufacturers' recommendations on shelf life and the best storage conditions. But many diet components, such as laboratory-processed plant materials or feed-grade ingredients, have limited or no technical data available. So the user is often forced to "take it or leave it."

Many analytical tests, standardized by the Association of Official Analytical Chemists (A.O.A.C.) in their Official Methods of Analysis (1975), are available to test the quality of dietary ingredients. But type, frequency, and amount of testing are generally dictated by what the insect is being reared for, type of program, number and qualifications of employees, and availability of space and equipment. If, for example, the diet is chemically defined and the insect reared is used in metabolic or nutrient-deletion studies, the dietary ingredients will have to be

tested extensively. But, if the insect is mass-reared for pathogen production on a crude diet containing plant material or minimally processed feed-grade material, fewer tests will be needed. Chemical analyses might indicate an adequate supply of an essential nutrient, such as an amino acid, but the processing may have caused nutrient loss or made some nutrients unavailable to the insect. In these instances, bioassays can be used after all processing and possible ingredient interactions have occurred. Finally, physical characteristics of the prepared media, such as pH, viscosity, color, and texture, can be checked.

Sudden changes, however minor, in ingredient quality may affect insect colonies dramatically. So, if the rearing program is sufficiently large or critical, all incoming shipments of ingredients should be tested. This testing could be especially important if suppliers or manufacturers are changed. In the future, specifications for nutrient content and product purity may be developed for insect diets. But, not having such standards, rearing programs must do careful testing.

Most commercial suppliers of food products, especially those used for human consumption, are regulated by the Federal Food, Drug, and Cosmetic Act administered by the U.S. Food and Drug Administration. The suppliers, in turn, maintain strict quality control in accordance with the A.O.A.C. These detailed and extensive analyses include protein content ($N \times 6.25$); amino acid composition; and mineral, vitamin, heavy metal, and microbiological profiles. Physical properties of the materials are also included in the technical data from the processors. Of these, size or fineness of the material is the major item. Other information includes weight per given volume (usually in cubic feet) or density, free-flowing quality of dry powder (sliding or bridging characteristics), type of container, and weight of standard packaging. The pH of single ingredients may also be critical to the acceptability of the final insect diet. For example, two methods of protein isolation (precipitation) are generally used in processing soy protein. One is acidic, producing a protein with a pH of about 5 (1:10 aqueous dispersion) and the other basic, with the protein having a pH of about 7. Depending on the quantity used, pH could significantly affect the activity of microbial inhibitors such as methylparaben (methyl *p*-hydroxybenzoate) or sorbic acid. Indi-

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vidual ingredients, especially alfalfa meal and wheat germ, should be monitored for chemical contamination from pesticides. Also, the ingredients should be checked for insect pathogens that could introduce disease into the colony. Further chemical analysis may not be needed except for the most stringent metabolic or nutrient-deletion studies.

Bioassay would probably be the chosen method of quality assurance for most insect-rearing operations. Bioassay is preferable to chemical analysis mainly because it measures how the ingredient affects the product (the insect) rather than simply defining the ingredient. And sophisticated equipment, highly trained technicians, and supportive laboratory space generally are not required for bioassays as they are for chemical analysis. Bioassay is also a measure of the insect's ability to digest and assimilate the nutrients. For example, Davis (1972) reported different growth rates in yellow mealworm, *Tenebrio molitor* Linnaeus, reared on casein obtained from four different suppliers. In such cases, both biological and chemical analyses would be required to determine if the amino acid composition was similar for like ingredients or whether methods of processing made one or more of the amino acids inadequate or inaccessible to the insects.

Common Sources of Diet Components

Proteins

Casein (minimum of 14.5% total dry nitrogen) is a common protein source in artificial diets for insects because it is highly purified and readily available. (See appendix for a comprehensive list of diet ingredients and some suppliers.) For example, according to ICN Nutritional Biochemicals' catalog for 1979/80, their entire manufacturing process is rigidly controlled, and the final product is subjected to biological and chemical assays. Maximums for total moisture, ash, and ether-extractable fat are met, as are specifications for total nitrogen (protein), amino acid profile (A.O.A.C. 1975), concentration of hydrogen ions (pH), and amount of lactic acid. The micrograms of B-vitamins per gram of casein are assayed and given on every batch or lot of Vitamin-Free Casein. Microbial limits are also established per gram of material (for example, standard plant count <5,000 colonies, coliform <50 colonies, yeast and mold <250 colonies, and *Salmonella* spp. and *Escherichia coli* negative). Apparently, bioassays are the most sensitive means of determining minor variations in the amino acid concentration of casein (Davis 1972) and other primary protein sources such as vegetable proteins and oilseeds that are deficient in one or more of the essential amino acids (Agricultural Research 1979).

Within the last decade, toasted, defatted soyflour (minimum of 50% protein) and cottonseed materials have been substituted for casein in *Heliothis* spp. diet. Purified mixtures of essential amino acids, plant extracts, wheat germ, animal lean meat and organs, egg albumen, and microbial protein concentrates have also been used to supply protein to the diet. According to the Archer Daniels Midland Co. (personal communication), heat treatment is used at various stages in the making of their soyflour toasted products. During this production, a protein dispersibility index is measured to insure the correct range or protein level (minimum of 50%) of the product before it is stored. Other tests done during these production steps may include analyses of moisture content, oil content, crude-fiber composition, urease activity, and particle size. Urease activity is a good indicator of trypsin-inhibitor level—the higher the level of urease, the greater the trypsin inhibition. In addition to routine chemical analysis, the soyflour also receives a microbiological assay similar to the one for casein. In diets based on wheat germ, both chemical and biological assays may be used on the wheat germ, which contributes protein and crude fiber to the diet.

Carbohydrates

Dextrose, sucrose, and fructose are added to the diet as major carbohydrate sources. Others may include honey, starch, cellulose, cereal grains, etc. Appropriate A.O.A.C. tests for types and amounts of sugar (both reducing and nonreducing) and starch materials may be run directly on these materials and on the wheat germ, since it also contributes carbohydrates.

Fats

Wheat germ, lecithin, cephalin, cholesterol and other sterols, grain oils, lard, and glycerol are probably the most common sources of fats in artificial diets. Some use purified essential fatty acids such as linoleic acid and linolenic acid. Wheat germ oil furnishes dietary lipids. Measuring the peroxide value of the extracted oil is an excellent method of determining its rancidity. According to Bio-Serv (personal communication), two widely used methods for determining the peroxide value (<70 milliequivalents/kg) are the active-oxygen method (Eastman Food Laboratory 1973) and a modification of the Wheeler (1932) method. Other grain oils, such as corn oil, are checked similarly.

The toasted wheat germ is also microbiologically assayed like the casein and soyflour (Bio-Serv, personal communication). Specifications for raw wheat germ and other natural ingredients, such as corn cob grits, soybean meal, etc., are not as rigid as they are for casein and soyflour. So the microbial load may fluctuate from 50,000 to 1

million spores/g, with a high incidence of coliform and fungal contaminants. As these high microbial loads are intrinsic to wheat germ, lots of raw wheat germ are generally accepted for industrial processing once they have passed rancidity tolerances.

Supplements

Salt or mineral mixtures, such as Wesson salts, are commonly added to the diet to supply the minerals needed for growth and development. These necessary ingredients are also found in wheat germ, ash of whole meat, fresh plant materials, and even water. Salt or mineral sources are analyzed quantitatively by appropriate A.O.A.C. tests, and some qualitative scanning for heavy metals and chlorinated hydrocarbons is also done.

Wheat germ, brewers' yeast, custom-formulated premixes, and mixtures of the B-vitamin complex are usually added as supplements. Chemicals such as ascorbic acid, choline chloride, and α -tocopherol are often added separately. Potency tests are conducted to establish pharmaceutical grade according to established procedures (Hoffman-LaRoche, personal communication). Techniques such as high-pressure liquid chromatography, which is quick and sensitive for such determinations, are described in the United States Pharmacopeial Convention's (1975) United States Pharmacopeia (U.S.P.) and A.O.A.C. Ascorbic acid has to conform to U.S.P. specifications (Bio-Serv, personal communication).

Chemicals such as mold inhibitors (for example sorbic acid), methylparaben, folpet³, benomyl⁴, propionic acid, phosphoric acid, Formalin (formaldehyde), and antibiotics are added to the diet in various combinations. Ingredients such as methylparaben must conform to the U.S.P.; sorbic acid must conform to the National Academy of Sciences' (1972) Food Chemicals Codex, and Aureomycin (chlortetracycline) must give a positive result for Qualitative Test according to A.O.A.C.

Another kind of supplement is food gum. It can be one of a diversified group of materials, including seaweed extracts such as agar, algin, carrageenan, and fucellaran; tree exudates and extracts such as gum arabic and tragacanth; seed gums such as guar and locust bean; cellulose derivatives such as carboxymethyl cellulose and microcrystalline cellulose; and microbial gums such as xanthan (Howell 1977). The function of these materials is also

diversified; for example, uses include adhesive and gelling (agar), bulking (gum arabic), suspending (carrageenan), swelling and inhibition of syneresis (guar), and binding (locust bean gum). Agar is typically analyzed by suppliers, harvesters, and manufacturers for moisture (16%–17% normal range), total ash (1.5%), acid-insoluble ash (0.07%–0.09%), pH (7.2), gel strength for a 1.5% solution (730–800 g/cm²), mesh distribution, and clarity of a hot 1.5% solution (Perny, personal communication). Also, a microbiological assay is done periodically; but microbes are rarely a problem for agar, which usually is negative for *E. coli* (0–10 range), has no *Staphylococci* spp., and has a standard plate count of 2,000–12,000 spores/g.

There are three commercially available plant-based agar types—*Gelidium* spp. (gelation point 34°–36° C), *Grassilaria* spp. (gelation point: 42°–45° C), and *Terocladia* spp. (not used widely; gelation point: 34°–36° C). The type of seaweed collected and even where it comes from affect the quality of extracted agar (Perny 1971). Most agar is currently processed outside the United States. The most common method of determining its gel strength is the Kobe test, which is performed on a 1.5% solution and measured in grams per square centimeters.) The Fira test measures deformation and fracturing of the agar, and gel strength determines the final firmness and resiliency of a completed diet.

Certain nonnutritive materials, such as alphacel and corn-cob grits, may be added to diets to increase bulk and replace some of the agar. For example, corn-cob grits (crude cellulose or fiber) were used to replace 80% of the agar in a casein and wheat germ diet for rearing *Heliothis* spp. larvae (Raulston and Shaver 1970). This filler material was monitored by the manufacturer for water absorption, capacity for carrying water and oils, capacity for holding water, and general physical properties (Foley 1978). These physical properties included bulk density, specific gravity, particle size, and flowability. Solubility in water and organic solvents was also tested. And elements such as structural and nonstructural polysaccharides were analyzed. Data were given for nutritive components such as protein, fat, crude fiber, ash, and vitamins, as were data for biodegradability, microbiological assay, pesticide residues, and combustibility.

Storage and Handling

There is probably no substitute for freshly processed ingredients, especially if they are perishable. But generally, a cool, dry area (for example, 15.6° C and 40% relative humidity) will suffice for prolonged storage. Some ingredients, such as wheat germ and soyflour, are packaged in large quantities (for example, 50- and 100-lb lots), so it is necessary to have adequate storage space. And some ingredients require refrigeration. Storage conditions should

³N[(Trichloromethyl)thio]phthalimide.

⁴Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate.

be designed to provide maximum shelf life for the ingredients.

Vitamin sources (or mixtures) and purified unsaturated fatty acids are among the most perishable ingredients used in insect diets. Refrigeration and desiccation extend the shelf life of vitamins; and fatty acids may be protected from peroxide formation by storing them in a 100%-nitrogen atmosphere. Wheat germ is also a perishable item that should be stored in a moisture-proof container; so it is often purchased in small quantities packaged in vacuum-sealed containers. Moisture-proof containers are recommended for storing most dietary ingredients—casein, sucrose, inhibitors, agar, minerals, etc.

Lot or batch numbers are provided by most suppliers, but receiving date should be placed on all incoming ingredients. If the ingredients are divided into smaller quantities or used to prepare premixed diet, the date received and lot number should be placed on all containers. Ingredient changes in the middle of a project should be avoided. Ingredients should be handled, mixed, ground, sifted, etc., in well-ventilated areas, because hazardous dusts may be produced. Equipment and machinery used in ingredient processing and handling should be easy to clean and disinfect.

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Appendix.—Insect-Diet Ingredients and Some of Their Sources

The list below gives commonly used diet ingredients not likely to be available locally. Each item is listed by non-proprietary name. Proprietary names and other manufacturer identifications, if any, descriptions, uses, amounts, etc. are given in parentheses. Names in brackets are short forms for manufacturers and suppliers. Full names and addresses, keyed to these abbreviations, follow the list. The main source for this information was Frass, the Insect Rearing Group newsletter.

Diet ingredients

- Acetic acid (glacial) [Baker].
Agar (fine) [Burtonite, Morehead], (granulated) [Bio-Serv], (type 100 cm) [Perny], several grades [U.S. Biochemical].
Alfalfa meal [Nutralite].
Alphacel (nonnutritive bulk) [ICN].
Ascorbic acid [Roche].
Beeswax (dark crude) [Sioux].
Benomyl (Benlate) [DuPont].
Bone flour (purified) [ICN]. Blended Food Product, Child Food Supplement, Formula No. 2 or CSM (corn, soy-flour, milk) [Krause].
Brewers' yeast (debittered) [Vitamin Food].
Calcium alginate (Kelgin HV) [Kelco].
Calco oil red dye (American Cyanamid's A-1700) [Cyanamid].
Carboxymethyl cellulose (sodium salt) [ICN].

β -Carotene (crystalline) [ICN].
 Carrageenan (Gelcarin HWG) [Marine Colloids].
 Casein (crude, 80 mesh; lactic, 30-40 mesh) [Milk Specialties, New Zealand, Erie], (Vitamin-Free) [ICN].
 Cephalin [ICN].
 Chlortetracycline soluble powder (Aureomycin; 25.6 g a.i./6.5-oz package) [Lederle, Ozark].
 Cholesterol (U.S.P. grade) [Bio-Serv].
 Choline chloride [ICN].
 Corncob grits (60 grade) [Andersons].
 Corn oil [ICN].
 Cottonseed meal (41% solvent extracted) [Yazoo], (Pharmamedia) [Traders].
 Cottonseed meats [Yazoo].
 Dextrin [ICN].
 Dextrose [ICN].
 Egg albumen [ICN].
 Ethanol [U.S. Industrial].
 Fibrin [ICN].
 Fish meal [ICN].
 Folpet (Phaltan) [Chevron].
 Formaldehyde (40% solution) [Fisher].
 Fructose (U.S.P. grade) [Bio-Serv].
 Fumaric acid [ICN].
 Gluten, wheat [ICN].
 Glutathione [ICN].
 Glycerol (chemically pure) [ICN].
 Glycogen (beef) [ICN].
 Guar gum [ICN].
 Gum arabic (acacia) [ICN].
 Inositol [ICN].
 KOH (45% potassium hydroxide solution) [Fisher].
 Lactalbumin [ICN].
 Lecithin [ICN].
 Linoleic acid (65% solution) [ICN].
 Linolenic acid (55% solution) [ICN].
 Locust bean gum [Bio-Serv].
 Maltose [ICN].
 Menadione (vitamin K₃) [Bio-Serv].
 Methylparaben (methyl-para hydroxybenzoate, technical) [Sigma, Tenneco 1, Tenneco 2].
 Milk powder (whole 28%) [Bio-Serv], (whole) [ICN].
 Penicillin G [ICN].
 Peptone [ICN].
 Potassium sorbate powder [Aceto-Chemical], (Sorbistat K) [Pfizer 1].
 Promine D (90%-95%, protein isolate) [Jeffards].
 Propionic acid [ICN].
 Propyl gallate (Tenox PG) [Eastman].
 Sorbic acid [Pfizer 2, Sigma].
 Sorbose (L form) [ICN].
 Soybean oil [ICN].
 Soyflour (toasted, Nutrisoy) [Archer].
 Soy protein (Supro 610, 100 mesh) [Ralston].
 Streptomycin sulfate [ICN].
 Tocopherol acetate (vitamin E, DL-Alpha) [Bio-Serv].

Torula yeast [Bio-Serv, ICN, St. Regis].
 Tragacanth [ICN].
 Vitamin premix (Roche No. 26862) [Roche].
 Wesson salts (mineral mix, also known as Salt W) [ICN], U.S. Biochemical], (modified) [Bio-Serv].
 Wheat bran [ICN].
 Wheat germ (raw) [Earthwonder], (raw, flaked) [Niblack], (toasted, flaked or regular) [Kretchmer].
 Wheat germ oil [Bio-Serv].
 Whole wheat flour [ICN].
 Xanthan [ICN].
 Zein [Bio-Serv].

Sources

Aceto-Chemical:
 Aceto-Chemical Co.
 Flushing, N.Y.
 American Cyanamid:
 American Cyanamid Co.
 Houston, Tex.
 Andersons:
 The Andersons
 Maumee, Ohio.
 Archer:
 Archer Daniels Midland Co.
 Decatur, Ill.
 Baker:
 J. T. Baker Chemical Co.
 Phillipsburg, N.J.
 Bemhem:
 c/o South Western Sales Association
 Jacksonville, Fla.
 Bio-Serv:
 Bio-Serv, Inc.
 Frenchtown, N.J.
 Burtonite:
 Burtonite Co.
 Nuttley, N.J.
 Chevron:
 Chevron Chemical Co.
 San Francisco, Calif.
 DuPont:
 E. I. duPont de Nemours Co.
 Wilmington, Del.
 Earthwonder:
 Earthwonder
 Springfield, Mo.
 Eastman:
 Eastman Chemical Products
 Kingsport, Tenn.
 Erie:
 Erie Casein Co.
 Erie, Ill.
 Fisher:
 Fisher Scientific

St. Louis, Mo.

ICN:
ICN Nutritional Biochemicals
Cleveland, Ohio.

Jeffards:
Doug Jeffards Co.
Nashville, Tenn.

Kelco:
Kelco Co.
Chicago, Ill.

Krause:
Krause Milling Co.
Milwaukee, Wis.

Kretchmer:
Kretchmer Products
International Multifoods Corp.
Minneapolis, Minn.

Lederle:
Lederle Laboratory
Dallas, Tex.

Marine Colloids:
Marine Colloids, Inc.
Springfield, N.J.

Milk Specialties:
Milk Specialties
Dundee, Ill.

Morehead:
Morehead & Co.
Van Nuys, Calif.

New Zealand:
New Zealand Milk Products, Inc.
Rosemont, Ill.

Niblack:
Niblack Foods
Rochester, N.Y.

Nutrilite:
Nutrilite Products
Lakeview, Calif.

Ozark:
Ozark Supply Co.
American Cyanamid
Kansas City, Mo.

Perny:
Perny, Inc.
Ridgewood, N.J.

Pfizer 1:
Pfizer Corp.
Chicago, Ill.

Pfizer 2:
Pfizer, Inc.
Doraville, Ga.

Ralston:
Ralston Purina Co.
St. Louis, Mo.

Roche:
Roche Chemical Division
Hoffman-LaRoche, Inc.
Nutley, N.J.

St. Regis:
St. Regis Paper Co.
Rhineland, Wis.

Sigma:
Sigma Chemical Co.
St. Louis, Mo.

Sioux:
Sioux Honey Association
Sioux City, Iowa.

Tenneco 1:
Tenneco Chemicals, Inc.
Organics & Polymers Division
Piscataway, N.J.

Tenneco 2:
Tenneco Chemical Co.
Chicago, Ill.

Traders:
Traders Oil Mill Co.
Fort Worth, Tex.

U.S. Biochemical:
U.S. Biochemical Corp.
Cleveland, Ohio.

U.S. Industrial:
U.S. Industrial Chemical Co.
Louisville, Ky.

Vitamin Food:
Vitamin Food Co., Inc.
Newark, N.J.

Yazoo:
Yazoo Valley Oil Mill
Greenwood, Miss.

Containerization for Rearing Insects

By Robert L. Burton¹ and W. Deryck Perkins^{2 3}

History

Many different kinds of containers have been used for rearing insects. The containers have been selected for their suitability and their availability from a multitude of widely used items. For example, Smith (1966) listed over 40 different containers used in many kinds of rearing programs, including milk bottles, battery jars, plastic dishes, metal trays, glass jars of all sizes, aquariums, trash cans, and barrels of various sizes (fig. 1). As the technology of insect rearing moved from natural diets to artificial diets, containerization became more standardized. Singh (1977) briefly surveyed 75 species from 50 families and 10 orders and found that 34% were reared in plastic cups; 22% in glass vials; 17% in petri dishes; 10% in fruit jars; and the remainder in test tubes, plastic or metal trays, flasks, plastic bags, plastic boxes or dishes, cardboard containers, salve cans, garbage cans, gelatine capsules, ice cream cups, multicell containers, and paper straws. The 1-oz polystyrene plastic condiment cup has been one of the more popular containers for use with synthetic diets. These cups serve well in both small and relatively large programs. They are fairly inexpensive, conveniently disposable, readily available, provide a good microenvironment, can be used in semiautomated equipment, and allow for isolation of cannibalistic species. Where species are not totally cannibalistic, another popular container, the paper drink cup, has been used for rearing insects in aggregate; these have many of the advantages of the plastic cups. Petri dishes have also been widely used for aggregate rearing. For axenic culture of insects, glass shell vials with cotton plugs have been used. For rearing stored-product pests on both natural and synthetic diets, glass jars of various sizes have been used.

In a few cases, containers have been designed and developed by researchers specifically for insect rearing. The need for such containers may result from cost, convenience, or species uniqueness. For example, Raulston and Lingren (1969), Morrison et al. (1975), and Hartley et al.

(1982) have developed multigrowth grids for lepidopterans. Peterson (1964) describes the design and construction of a multitude of cages and containers used specifically for insects. Other specific rearing containers are described by Baumhover et al. (1977), Harrel et al. (1973), and Leppla et al. (1975).

As the technology of insect rearing moves forward, the adequacy of the rearing container will be examined more strenuously. Improvement of insect containerization requires an indepth look at the related aspects of container design. This paper discusses these aspects and other considerations that might be needed in developing or improving an insect-rearing container.

Design and Selection of the Rearing Container

General considerations

An effective container must protect the food; present the food to the insect in an acceptable manner; provide the proper surfaces and atmosphere for the insect; and confine, and, in some cases, separate cannibalistic insects. Above all else, the rearing container must meet the physiological and ecological needs of the insect whether it be environment, space, or other. At the same time, the container must have certain general structural qualities and specifications that conform to the activities of the insect-rearing program such as filling, implanting insects, storing, handling, and cleaning.

Physiological and ecological considerations

Size, shape, and surface.—The size of the container affects the insect in several ways. If wandering by larvae must be prevented or reduced, the container must hold the insect close to the diet. Population density, which depends partly on container size, can affect such factors as growth and fecundity. All containers must provide enough space to permit unrestrained larval development and pupation. Container size is important in rearing partly cannibalistic species because sufficient space reduces this activity. When insects can be reared in aggregate, each species probably has an optimum container size.

The shape of a container is also important. Shape may affect diet thickness or the ratio of total volume to surface area of the diet and thus influence moisture retention. Shape may affect pupation sites and access to the diet.

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Figure 1.—A variety of containers that have been used for rearing insects.

Proper surfaces must be available for unrestrained molting and pupation. For example, in many container types, the unwaxed closure surface has traditionally provided a surface for these activities. Both size and shape affect air and moisture exchange, because air movement around and between containers may be important for proper ventilation.

Air and moisture exchange.—Air and moisture exchange may be the most important physiological and ecological function of the rearing container. The insect must have a favorable microenvironment, which can be produced only if ventilation and moisture regulation in the container meet the insect's maximum respiratory demands (these may be difficult to determine) and moisture requirements (which will vary among species). Controlled water-vapor transmission can regulate the drying rate of the diet. Also, and usually more important, it can eliminate excessive moisture buildup in the container. Free moisture promotes spore germination and growth of unwanted microbial contaminants and is one of the primary causes of these epizootics. Traditionally, the closure has provided

the ventilation (see "Closure Requirements") since the container must contain the wet diet and thus be resistant to water movement. Common techniques for air and moisture regulation have been the use of closure materials that ventilate (such as unwaxed paperboard) and regulation of the size of a ventilation area covered with screen, fabric, etc. Conditions outside the container then become important in the regulation of moisture in the container. Moisture requirements of the microclimate vary from species and among the various life stages of the insect and require that optimum levels be known for each insect to be reared.

Toxicity.—Certain plastics, such as cellulose acetate, can be toxic to some species of insects (Chada 1962). Also, some types of wood may be harmful. Some paints, varnishes, and preservatives have toxic properties, especially when freshly used. All these substances can also cause toxic reactions in plants and thus affect the insects if the plants are used as hosts. Being aware of possible toxic effects is important, then, in choosing container materials.

Table 1.—Characteristics of materials for making rearing containers

Material	Cost	Durability	Weight	Advantages	Disadvantages
Paperboard	Low	Low	Light		Wears quickly; its chaff is harmful; unstable to all liquids if unwaxed.
Metal	High	High	Heavy	Rigid, strong	Does not hold finishes well; oxidizes; sharp corners and edges hazardous.
Fiberglass	High	High	Light	Rigid, strong, easily cleaned.	Prone to chipping and cracking and may irritate skin.
Pressed fiberboard.	Medium	Medium	Medium		Short-term moisture and oil resistance.
Wood	High	High	Medium	Easy to make at rearing facility.	Possible toxic properties to insects in some woods, pre-finished woods, and various finishes.
Thermoplastics:					
Styrene	Low	High	Light	Good for small cups and boxes; ideal for disposables.	Brittle.
Polyethylene	Low	High	Light	Resistant to most solvents, oils, etc; high density; low maintenance.	Static charge attracts dust.
ABS	Medium	High	Medium	Relatively rigid; good solvent resistance; easily fabricated.	None.
Cellulose nitrate.	Medium	High	Light	Transparent; easily fabricated; good for plant-insect cages.	Inflammable.
Polycarbonate film.	Medium	High	Light	Transparent; easily fabricated.	Inflammable.

General structural and physical considerations

Size and shape.—Size and shape must also be considered in terms of structural design and container processing. Size and shape can affect container strength. Size is important for handling ease in terms of hand-load capacity. Size may also affect storage efficiency, and shape certainly does. Stacking, nesting, hanging, etc. need to be considered, especially as ways to conserve expensive controlled environmental space during incubation and to provide proper air circulation about the containers. Size and shape must conform to processing equipment used in

the rearing program; close tolerances may be required by automated equipment. Closures, seams, etc. must fit properly to prevent airborne contamination of the contents, excess moisture loss, and escape of the insects.

Durability.—In the case of reusables, durability is important in reducing replacement costs. Reusables must be cleaned, so containers must resist wear due to repeated cleaning, which may include both strong cleaning solutions and steam heat. Reusable containers that can withstand sterilization temperatures might be preferred if disease or other contamination problems are anticipated. Containers should also be resistant to appropriate diets; some are

Table 2.—Cost factors to consider when selecting an insect-rearing container

Cost element	Design effect	Container type	
		Disposable	Reusable
Purchasing	Mass-produced containers less expensive; prefabricated custom-made more expensive. Initial design important to prevent costly die changes.	Low initial investment; continuous costs accumulative; price increases a problem.	Initial investment high but little additional costs. Initial design critical.
Shipping and storage . .	Costs reduced with good nesting and stacking qualities. Custom-made containers may add to shipping costs.	Costs high and continuous	Shipping costs low; perhaps less storage required.
Handling	Properly designed containers promote handling efficiency and reduce damage.	Few handling problems	Much handling required.
Maintenance	Good design promotes stability	No costs	Some maintenance and replacement costs.
Cleaning	Well-designed containers more cleanable.	No costs	Labor, cleaning supplies, and cleaning area costly.
Disposal	Type of material important	Disposal costs high; environmental impact important; may require compaction equipment; recycling possible.	Minimal disposal costs.
Availability	Commonly used prefabricated types more available.	Dependent on industry; inventory critical.	Less dependent on industry.

dispensed hot and wet and, in some cases, have corrosive properties. Disposables need not be durable.

Visibility and accessibility.—Container contents must be easily seen. Frequent visual inspection is extremely valuable to program maintenance and quality control. Also, if host plants are used, the container must allow adequate light transmission for the plant. Efficient removal of insects from the containers requires good accessibility. Both visibility and accessibility save valuable time when insects are removed from containers at various growth stages.

Availability.—Containers must be readily available for programs with continuous production. (See appendix for a list of some commercial sources for containers and materials.) Close inventories and projected needs are especially necessary for disposables. Mass-produced and widely used containers, those primarily used for other purposes, such as paper drink cups, are generally more reliably available for future needs than customized containers that are in less demand. Such prefabricated availables also provide a developing program with a very large choice of sizes, shapes, materials, and prices. Users of handmade containers should consider continued availability of materials.

Materials.—Materials for rearing containers are relevant to almost every design criterion and every rearing requirement. Some of the more common materials available for container fabrication are listed in table 1 with characteristics that may affect container design.

Cost.—The rearing container may be the most costly part of the rearing program. Costs of disposables reflect direct purchases, and costs of reusables reflect expensive cleaning and the eventual replacement, which will also be expensive. Size of the rearing program generally affects the cost per container per insect, as larger quantities generally produce lower unit costs. When programs become large enough, equipment for container fabrication becomes more economically realistic. But fabrication machinery requires large initial cash outlays that are sometimes difficult to justify, so proven container design is especially important. The relationship of container design to container requirements also affects container costs as does the choice of disposable or reusable containers (table 2). Rearing technique may dictate which type of container to use; but, in other cases, careful consideration should be given to cost.

Closure requirements

Obviously, the container and the closure should be considered as a unit, although there may be several combinations to choose from. So factors affecting container choice also affect closure choice. Because the closure is usually the part of the container that provides ventilation, and therefore is a device for controlling air and moisture exchange, it is especially necessary to be aware of the complex relationship between container and room environment when choosing closure types and materials. Simple materials, such as screen or cloth used to cover vents, cardboard caps, or cotton plugs are often all that is needed for adequate ventilation with proper room conditions. Different structure and compactness of materials used for closures can affect the rate of evaporation from the rearing container. Burton (1967) used unlined (unwaxed) cardboard caps when rearing fall armyworm, *Spodoptera frugiperda* (J. E. Smith); Raulston and Lingren (1972) used polypropylene cloth on multicell units for rearing tobacco budworm, *Heliothis virescens* (Fabricius); Hartley et al. (1982) used rigid sheet polypropylene on multicell units for rearing *Heliothis zea* (Boddie); Kogan (1971) used layers of Cellucotton wadding to aid in moisture removal from rearing cells for Mexican bean beetles, *Epilachna varivestis* Mulsant; and Harrell et al. (1977) used Tyvek for rearing the boll weevil, *Anthonomus grandis grandis* Boheman. A special requirement for cap thickness is also necessary to prevent the corn earworm from chewing out. The use of machines (Burton and Cox 1966, Davis 1982) for manipulating closures adds still another dimension for required tolerances.

The need for access into the rearing container is an important factor in choosing closures. More durable material and special handling techniques may be needed when closures have to be reused as insects are observed and fed during development. Ridgway et al. (1969) described a method for access into Hexcel rearing containers for repeated feedings. Cardboard lids on plastic cups have proven useful when repeated feeding is desirable and have also been adapted by Harrell et al. (1968) to mechanical harvest of the fall armyworm, and similarly by Davis (1982) for the southwestern corn borer, *Diatraea grandiosella* (Dyar). Some methods of harvest require materials with special characteristics. For example, Harrell et al. (1974) built a machine to remove Tyvek from multicell rearing trays. Tyvek, a durable plastic product, provided sufficient strength for efficient operation of the machine, while paper cover material, weakened by moisture from the diet, tended to pull apart under stress.

Close tolerances for closures are important. To prevent closures in some types of containers (for example, lids for plastic cups) from being pushed out or from falling off, the container manufacturer's specifications for closure

diameter requirements should be followed. Storage conditions may also affect the degree of fit for fibrous closures by causing swelling under moist conditions and shrinkage in drier environmental rooms where containers are kept during insect development.

Technical information is seldom used but could be valuable in selecting the proper materials for closures and containers. For example, several give characteristics of paper products, such as the porosity by resistance to airflow and the water-vapor transmission rate. The Technical Association of the Pulp and Paper Industry (TAPPI) provides official test methods and respective TAPPI reference numbers to member firms. These numbers provide a standard that can be used when characteristics of paper products are of interest.

The Future of Containerization

As costs continue to increase and demands for more and higher quality insects grow, efficiency in rearing and in controlling rearing variables and a need to standardize programs will become more important. Use of the 1-oz plastic cup has been so successful in most rearing programs that a change here will probably not occur soon. This slowness to change may also be true for other types of disposables. But certain trends such as inflation and scarcity of petroleum products will continue to have their effect. These two factors may well bring about the gradual end to the disposables we now so conveniently use. When this end occurs, the need to develop replacement reusables will be even more important, and better designs and more convenient reusable containers may be the future of insect containerization.

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Appendix.—Some commercial sources for containers and materials

Containers and materials	Use	Sources
Paper containers for food and drink.	Insect holding	Solo Cup Co., Urbana, Ill.; Sweetheart Cup Div., Owings Mills, Md.; Lily-Tulip Div., Toledo, Ohio; Dixie Cup Div., Green Bay, Wis.
Plastic stock	Forming film for larval rearing.	Standard Packing Corp., Clifton, N.J.; B. F. Goodrich Co., Atlanta, Ga.; Foster Grant Corp., Sandusky, Ohio.
Tyvek and paper stock plastics.	Cover of rearing cells.	Allegheny Label Inc., Cheswick, Pa.; Cellu-Craft, Inc., New Hyde Park, N.Y.; Tolas Corp., Philadelphia, Pa.; Transilurap Inc., Cleveland, Ohio.
Porex polypropylene sheet.	Cover of rearing cells.	Porex Materials Corp., Fairburn, Ga.
Polypropylene cloth fabric.	Cover of rearing cells.	Chicopee Manuf. Co., Cornelia, Ga.
Polystyrene cups.	Larval holding	Unijax, Inc., Raleigh, N.C.; Premium Plastics, Chicago, Ill.
Paper lids	Cup cover	Unijax, Inc., Raleigh, N.C.; Premium Plastics, Chicago, Ill.; Standard Cup & Seal, Chamblee, Ga.
Polycarbonate plastic film.	Cage construction	Cadillac Plastic & Chemical Co., Oklahoma City, Okla.

Section 3

Engineering for Insect Rearing

Tremendous progress has been made in the last few years in the art of controlling insects. Most of this work with insects has rightly been considered the province of entomologists. But engineers with the proper background and training can provide important expertise for solving some insect-related problems, and they are making significant contributions.

Engineering involvement on many of the insect-related problems has been slow to materialize. A few engineers have been working for many years with entomologists on specific problems of mutual interest, such as trapping and insecticide application. Recently, other engineers have begun working on rearing and modeling. There are still many more engineering problems related to insects than there are engineers working to solve them. So only a few of the major problem areas will be covered in this section—environmental control for insects and personnel, control of respiratory hazards to humans, design of insect-rearing facilities, insect-rearing automation, materials-handling processes, quality-control procedures, and systems analysis and modeling. Many other areas have problems needing engineering attention—shipping; harvesting insects; packaging; diet preparation; design of rearing containers and covers; safety; building materials; quality and intensity of light; photoperiod; thermoperiod; work areas; rearing systems for parasites, predators, and pathogens; and specific rearing systems for different insects.

Adequate engineering involvement in all the problem areas would require additional personnel. Those already involved are spread too thin, and the probability is high that the number will decrease. There are too many problems for a reduced number of qualified personnel to handle. A person's academic training in engineering or entomology does not fully qualify him, nor does it guarantee success. This type of training is essential, but it must be supplemented with experience and knowledge gained from a hands-on operation.

Personnel already at work could probably be used more effectively by the establishment of a central insect-research center staffed with qualified engineers. These engineers could be assigned to seek solutions to the most pressing problems as they arise anywhere in the United States. If a problem could not be solved at the center, then the staff would go to the problem area, solve it, and return for further assignment. Also, the staff could supply a much-needed consulting service. In a few years, such a center, properly staffed and supported, could probably make tremendous progress toward solving some of the problems, at much less cost than otherwise. A review of recent accomplishments indicates that progress has been rapid when engineers and entomologists cooperate, as they complement each other well.

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Controlled Environments for Insects and Personnel in Insect-Rearing Facilities

By Charles D. Owens¹

Introduction

Environmental control involves regulation of the external conditions that affect the growth, development, and behavior of an organism. In a facility where insects are reared, particularly in large numbers, the conditions that must be regulated include temperature, humidity, noise, and the movement and cleanliness of air. Experience has shown that as the insect concentration increases in a production system so does the incidence of contamination, disease, and related problems. Mass rearing often causes allergy problems for workers; therefore they need protection from excessive exposure to moth scales and urticating or allergenic setae (Etkind 1976, Press et al. 1977). And rearing under unsanitary conditions can result in insect death because of microbial contaminants or pathogens, and workers are likely to become sensitive to these allergens. So provision of clean air and maintenance of sanitary conditions are especially important in the design of a mass-rearing facility. In fact, all aspects of the environment in a mass-rearing facility can be made to perform satisfactorily with proper engineering design. This paper surveys equipment and design factors important to control of rearing environments for both insects and personnel.

Air Cleaners

Air must be clean before it is brought into a rearing room. And undesirable airborne particles and gases must be removed from air that is recirculated. Equipment to clean or filter air includes: air washers, viscous fiber or dry filters, electrostatic precipitators, and cyclones. A more detailed description of air cleaners and air performance can be found in the handbooks of the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (1972, 1973, 1975, 1978).

Air washers are dust-collecting devices that pass the air through a spray of water to wash out the contaminants. They are used to control temperature and humidity in environmental rooms. But they are relatively inefficient in

removing fine particles, so they are used primarily to remove undesirable gases and wettable particles.

Viscous fibrous filters are flat panels of coarse fibers. The fibers are coated with a viscous substance that acts as an adhesive to the impinging particles. Some of these filters can be cleaned and reused, and some are disposable.

Dry filters are usually made of fiber- or blanket-like material of varying thicknesses. The removal of airborne particles depends on the closeness of the fibers. Industrial cloth bags are used as filters for air having a high dust load with a particle size of 0.5 μm or larger. The efficiency of industrial filters varies and is rated according to the specifications of the U.S. National Bureau of Standards or standard dust-particle test. Air filters used in ventilating systems are usually rectangular dry-fiber units. Absolute or HEPA (high-efficiency particulate air) filters are used for very low dust loads and have a high efficiency for collecting particles down to 0.3 μm . They are rated by a smoke test known as the DOP (dioctyl phthalate) penetration test. The HEPA filters are used for cleanrooms and laminar-flow hoods.

Dry centrifugal collectors called cyclones are the most common type of collector for large particles. They separate particles from the air by radial acceleration or centrifugal force. Air entering the cyclone is transformed into a vortex, causing centrifugal action to force the particles against the wall. Ultimately, the particles travel to the lower end of the cone where they are collected. Cyclone air cleaners are generally used when particles are present in large quantity and their size exceeds 50 μm (for example, moth scales). Although some facilities use a series of cyclone collectors to remove scales from the air, a cyclone combined with a bag filter is more efficient.

Electronic air cleaners used in cleaning ventilated air are designed as electrostatic precipitators. The air passes through a high potential ionizer field that gives a charge to the particles; these are then attracted to ground plates. The particles can be removed from the collector plates by washing. Since the charged particles form clumps on the plates, regular cleaning is necessary.

Selection of an air filter depends on the amount and size of contaminants in the air and requirements for air cleanliness. As more insects are reared, the need for clean air

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Table 1.—Number of particles ($>0.5 \mu\text{m}$) per 0.03 m^3 of air outside and inside the insect-rearing facility at Otis Methods Development Center

Location	Filter	Average particle count ¹
Outdoors	None	50,000
Laboratory	None	90,000
Holding room:		
1½ minutes per air change	95% HEPA	200
3 minutes per air change	95% HEPA	500
Infesting room workroom	95% HEPA	1,500

¹Reading taken with particle counter.

increases because of the increased occurrence of pathogens and microbial contaminants. If the insects are highly susceptible to airborne pathogens, the 99.9% HEPA filters should be used with at least one air change every 2 minutes. Generally, the 95% HEPA filter (hospital grade) will maintain sufficiently clean conditions in insect-rearing and other work areas (table 1). Also the more efficient the filter, the greater the air pressure differential across the filter. So high-efficiency filters require care in installation to prevent air bypass leaks.

Controlling Environment for Insects and Personnel

Control of air, temperature, humidity, and other environmental factors is required in all areas of an insect-rearing facility, but how much control and how it is accomplished vary for different areas and insects. And, unless insects are held for prolonged periods in workrooms, control of some or all of these factors in these rooms can be designed for worker comfort.

Controlling air for personnel

Because dust generated from the body scales of adult moths is a primary cause of human allergies, the air should be free of contaminants in areas where workers must handle insect stages that are potentially hazardous to their health. So, during handling of insects, the air should flow past the workers, over the work area, and through a filter system before returning to the room. A velocity of 30–40 m/min is required to capture and convey the scales and hairs. A velocity of 15 m/min or higher will transport the smallest ($\leq \mu\text{m}$) and lighter particles.

The easiest way to protect the worker is to use a work-table or clean-air station, such as the one developed for handling larvae and pupae of the gypsy moth, *Lymantria dispar* (Linnaeus), (fig. 1). Air is drawn past the worker,

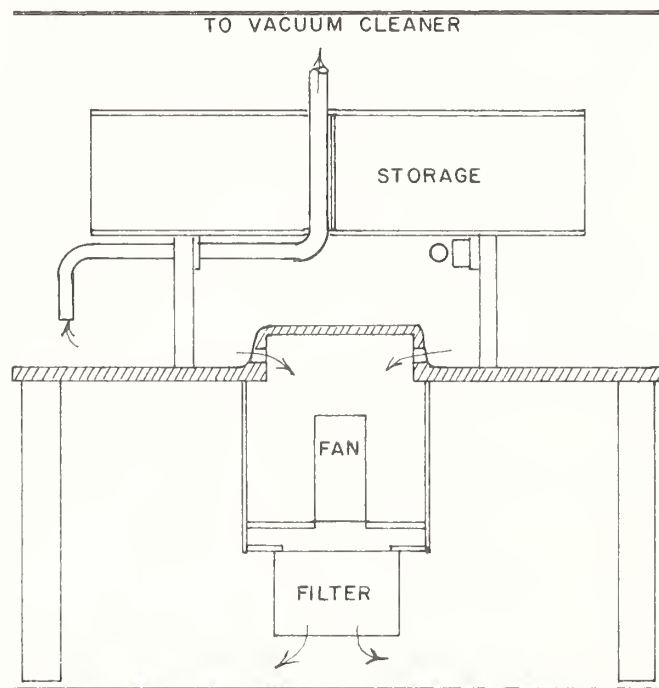
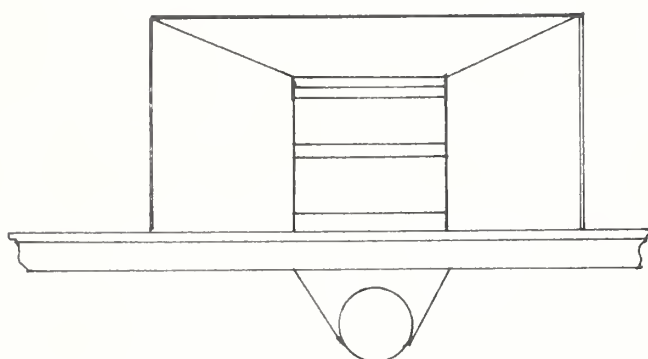


Figure 1.—Cross section of a clean-air work-table.

into slots in the center of the table, and through an industrial-grade filter; it is then discharged back into the room. The industrial filter has a glass-fiber pack for the prefilter and an overall efficiency rating of 80%. The air velocity, varying from 15 m/min near the workers to 107 m/min at the opening, causes the air current to flow toward the center of the table. Because of the open design of the system, large particles settle on the table and are removed with a separate vacuum system. Two vacuum outlets connected to one central vacuum cleaner can remove particles such as webbing, cast skins, and clumps of scales when both outlets are operating. For small operations, commercial clean-air hoods can be used if the air current is drawn into them; most horizontal laminar-flow hoods (a kind commonly used in laboratories) blow clean air out toward the operator and are not suitable for small operations.

Disposal of live moths requires a relatively high air velocity that can be provided by using a hood that tapers toward a slot (fig. 2). The air velocity should exceed 30 m/min at the front and range from 305 to 457 m/min at the exhaust. With this type of hood, the front part is used to transfer or handle moths, and the area nearest the discard slot conveys moths or other heavy particles into the vacuum. This arrangement is used in the adult-handling room of the gypsy moth rearing facility at the



HIGH VELOCITY HOOD

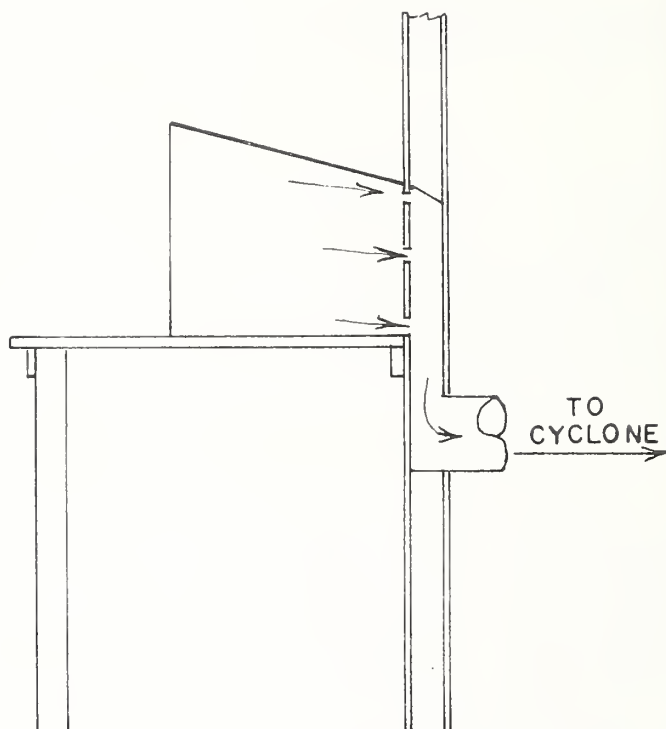


Figure 2.—Diagram of a high-velocity work hood connected to a cyclone collector.

Otis Methods Development Center, Otis Air National Guard Base, Mass., for housing mating cages, removing excess moths, and handling egg masses. The high-velocity outlet is connected to a cyclone that collects the moths, while the lighter particles are removed by a bag filter. Before the air returns to the room, it is passed through a 95% HEPA filter (Bell 1981). Some rearing facilities have moth cages placed over funnels that are connected to a cyclone air cleaner. This arrangement also extracts the scales that become dislodged from the moths and reduces the number of airborne particles. In addition to the clean-air work stations, each workroom should have all air pass through a HEPA filter at least once every 3–4 minutes.

Controlling air for insects

HEPA filters are necessary to reduce the incidence of airborne bacterial, fungal, and viral pathogens, thereby decreasing the probability of diet contamination and disease. Also, increasing the number of air changes per unit of time (for example, to 2–3 minutes per change) generally decreases the number of particles. A slight positive pressure should be maintained in larval-development rooms so that contaminated air will flow out when the

doors are opened. Positive pressure is accomplished by having a filtered fresh-air intake. Larval-development rooms do not require much outside air, and the more times air passes through a filter, the cleaner it should be. Rooms that are normally contaminated can be operated under pressure so air will flow into them (Shapiro 1980). Negative pressure is accomplished by using an exhaust fan to exhaust air through a HEPA filter. The air in insect-rearing rooms must be controlled so that the temperature, humidity, and air velocity are fairly uniform. This uniformity is difficult to accomplish.

Air distribution can be provided by standard heating and ventilating arrangements in work, diet-preparation, and insect-handling areas. But holding rooms require a system that produces more uniformity. Standard vertical laminar-flow cleanrooms have the air blowing through a perforated ceiling to a grated floor. This design is expensive, and keeping the area below the grating clean is difficult. A better arrangement, adapted from a design by Klassen (1971), provides good air distribution by using a wall plenum return and a perforated ceiling to allow air into the room (fig. 3). Moving the air horizontally, as in a clean-air, horizontal laminar-flow system (Harrell 1979b), also provides superior air distribution. Air enters through

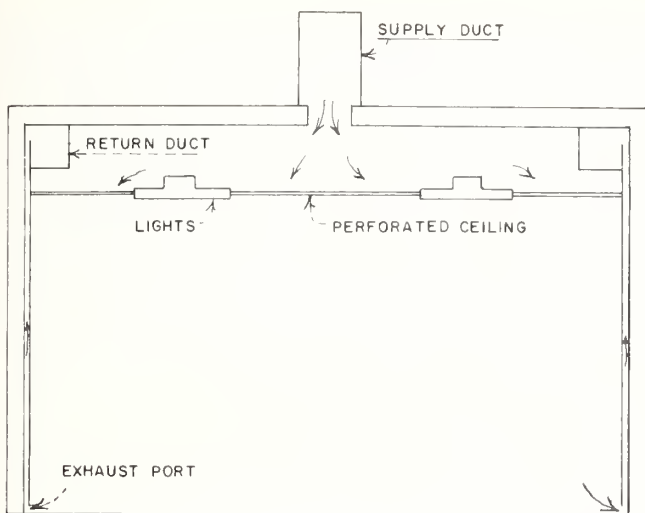


Figure 3.—Diagram showing airflow in a larvae rearing room.

holes in one sidewall and exits through a similar arrangement on the opposite side. This design works better in narrow rooms.

A 2-3 minute air-exchange rate requires more airflow than is needed to maintain the temperature and humidity. Therefore, to conserve on installation and operating costs, the air-handling system may be designed to allow 50%-75% of the air to bypass the conditioning equipment (fig. 4). This bypass can be accomplished by making the duct area larger than the heating and cooling coils or by locating the conditioning equipment in a bypass parallel to the main duct. The latter arrangement was used effectively for the Otis Methods Development Center's gypsy moth rearing facility. With the bypass system, the air-conditioning equipment was sized to the heating and cooling load and not to the airflow rate. This arrangement was less expensive than a system without a bypass would have been and provided better temperature regulation.

Controlling temperature and humidity

The longer the larval growth period, the more nearly uniform the temperature has to be for developmental synchrony. In a large room, the temperature can be controlled by a good ($\pm 1^\circ\text{C}$ or better) thermostat. But small cabinets or walk-in chambers require thermostats with sensing devices that respond more quickly to changing environmental conditions. Since energy requirements are high for large larval development rooms, it is more economical to have a $\pm 2^\circ\text{C}$ differential between the heating

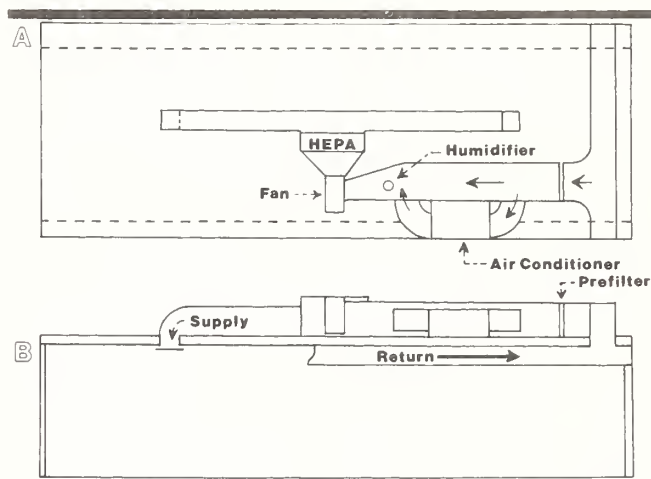


Figure 4.—Typical arrangement of air-conditioning and filtering equipment in a larvae room. A. Air-conditioner bypass system. B. Oversized duct system.

and cooling cycles rather than the typical on/off control system. A regular fluctuation ($1^\circ\text{--}2^\circ\text{C}$) in room temperature does not hamper insect development. One way to compensate for differences in the room environment is to routinely rotate the insects around the room so that all are exposed to similar conditions. This rotation can be done with a continuous conveyor system.

The temperature and humidity of the room are not necessarily the same as they are in the rearing container. But the room environment affects the diet's drying rate (Hare et al. 1973). The amount of diet, number of insects per container, the material the containers are constructed from, and density of containers also affect humidity and air exchange. Therefore, room conditions should be adjusted for all these influences so that the desired environment will be maintained in the container (Harrell 1979a).

The best way to add humidity to the environment is to inject steam into the airstream in front of a recirculating fan. Spray or atomizer devices often discharge excess water into a room and become clogged with mineral deposits. And the hot-air-furnace humidifier is not satisfactory in insect-rearing facilities because it requires a large heat differential to operate, and that is not present in a rearing room.

The cooling coils of air-conditioners can be used to dehumidify some rooms. Low airflow and low coil temperature are required to maintain a low dewpoint temperature. So large refrigeration units with special compensating devices are used to avoid frosting problems. If humidity

is to be held below a 10° C dewpoint, some type of absorption dehumidifying system must be used. The most common system uses silica gel or activated alumina as the desiccant. Generally, the equipment incorporates a regeneration system to reactivate the desiccant.

Controlling lighting

Work areas need nonglare overhead lighting; low-level general illumination is adequate for holding rooms. Light intensity experienced by the insects will vary considerably depending on types of containers and on the way the containers are distributed in the rooms. Generally, insects respond to very low light intensity, so just enough is provided to entrain their rhythms. Supplementary lighting of 50–100 fc is required for tasks such as handling newly hatched larvae or counting eggs. This lighting is similar to the requirements of desk and office work. The placement, shielding, and type of light, and also the texture of work surfaces, can affect the amount of eyestrain caused by glare or improper illumination.

Controlling noise

In rearing and handling rooms, noise is produced by mechanical devices and high-velocity blowers. An airflow system has two sources of noise—the low frequencies from the fan and the high frequencies produced by air flowing through the outlet or inlet grills. Centrifugal fans with airfoil blades produce less noise than other types. Sound transmission can be reduced by isolating the fan and duct with a flexible coupling. HEPA filters also reduce noise levels. Designing ducts and air outlets so that air velocity is less than 200 m/min reduces the high-frequency sound generated by grills. Increasing the mass and stiffness of hoods will dampen their vibrations. Sometimes, as with the high-velocity exhaust hood used in moth rooms, the noise level cannot be reduced to desired levels. In such cases, either the length of exposure should be reduced or earplugs should be used. If the sound is annoying, but not hazardous, structural changes can be used to shift it to a less objectionable frequency, or a more pleasant sound can be added to mask the original. Noise can also be reduced by decreasing the energy that drives the vibrating components, changing the coupling between the energy source and the acoustical radiating system, altering the structure that is radiating the sound, and using attenuators.

Other Design Considerations

Operating costs, as well as initial costs, must be considered in designing a facility. For example, more energy is required to move air through a more efficient filter. If industrial-grade prefilters are used, HEPA filters will last longer. Increasing the number of air changes per unit of

time requires more energy and increases the noise level. Rooms should be well insulated (R-11 or better) to reduce the amount of air-conditioning needed and to moderate temperature changes during electrical power outages. The walls, floors, and ceiling should be finished with materials that facilitate cleaning and sanitizing (smooth and washable surfaces such as concrete floors and painted walls and ceilings). A central vacuum-cleaning system or a portable cleaner with an absolute filter should be used to remove dirt from the floor. Workers should take precautions to prevent transporting contaminants into the facility. And the work should be performed in a sequence ranging from clean to dirty.

Recommendations

To best control the environment of insect-rearing facilities: Provide individual air-conditioning units and controls for all rooms; have a backup holding room, so insects can be moved in case of mechanical failure or contamination; insulate rooms to reduce temperature change during an electrical power failure; properly install HEPA filters in all rooms; use industrial-grade filters as prefilters to the conditioning equipment; recirculate the air every 2–3 minutes; maintain low air velocities to reduce noise; provide uniform airflow in the rearing rooms; distribute rearing containers so airflow affects them equally; use steam humidifiers; install exhaust hoods equipped with clean-air filters; and monitor air cleanliness with a particle counter.

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Controlling Respiratory Hazards in Insectaries

By Wayne W. Wolf¹

Introduction

Present laws in this country mandate that the employer must provide a safe working place for employees. The first responsibility lies with the employer in recognizing hazards and providing appropriate equipment and work procedures. Further responsibility lies with supervisors and workers in providing instruction, maintaining equipment, and performing work in a safe, prescribed manner.

The objectives of programs for respiratory protection are to save workers from suffering ill effects, prevent lost time, and prevent permanent injury. These objectives may be accomplished by recognizing that respiratory diseases can be prevented like other diseases. For a disease to occur, there must be a susceptible host, a means of transmitting the disease, and a successful transfer. Preventing transmission will be the primary defense against respiratory diseases in an insectary.

Respiratory Hazards in Insectaries

The respiratory hazards often present in insectaries are particulate matter and gases that adversely affect the human body. These hazards may originate from various insect stages, insect waste products (such as frass), mold spores, materials used in insect diets, or cleaning and sanitizing chemicals. These substances have various modes of action and may produce skin injuries, allergic reactions, asphyxiation, or damaged internal organs. Allergic reactions may involve the skin, eyes, and respiratory tract, and prolonged exposures may produce irreversible pulmonary damage. Allergic reactions may develop after repeated exposure over a period of years, and some individuals may become very sensitive to low concentrations. Routine work such as counting, sexing, dissecting, marking, weighing insects, and handling and cleaning insect cages can increase the air contamination. Moth scales are a primary source of contamination.

Particles greater than $2\frac{1}{2}$ – $3\text{ }\mu\text{m}$ in diameter are mostly deposited in the upper respiratory system, while particles about $1\text{ }\mu\text{m}$ in size are deposited most efficiently in the

alveolar cells of the lungs. Conditions such as rhinitis, laryngitis, and bronchitis can develop in the upper respiratory system. In the lower system, conditions such as emphysema, pleurisy, pneumonia, and pneumoconiosis can develop (Olishifski 1978).

Airborne concentrations of substances that workers may be safely exposed to day after day and the acceptable conditions of exposure are called threshold-limit values (TLV). The TLV guides that have become the most widely accepted are those issued by the American Conference of Governmental Industrial Hygienists; they are reprinted in National Safety News (1979a). The TLV's listed include various chemical and physical hazards that may be encountered in an insectary. Hazards such as insect scales or allergy-inducing materials are not covered.

Detecting Contaminants in Insectaries

Assessing the severity of hazards or effectiveness of control equipment requires some method of detection or measurement. Measurement of many gases can be accomplished quickly and inexpensively with small "grab" samplers. Such a sampler may consist of a small glass tube and a pump that forces a measured quantity of air through the tube. The tube contains granular chemicals that change color to indicate the concentration of a specific gas such as chlorine or formaldehyde. Sources of sampling equip-

Table 1.—Particle size distributions in various locations at the Western Cotton Research Laboratory¹

Particle size (μm)	Distribution (particles/ m^3)		
	Air in pink bollworm cages ²	Outdoor air ³	Filtered air in moth room ⁴
0.5–1	6,600,000	1,400,000	74,000
1–2	2,600,000	560,000	16,600
2–5	1,600,000	233,000	14,800
5–10	350,000	7,800	5,300
>10	2,900,000	3,500	9,200

¹Measured with Climet Model 250 particle counter.

²Average of 8 cages, one run per cage.

³Average of 6 runs.

⁴Average of 12 runs (3 runs every 4 hours) after room reached equilibrium. Filter system shown in figure 4.

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ment may be obtained from offices of the U.S. Occupational Safety and Health Administration (OSHA) or from various safety-product buyer's guides such as National Safety News (1979b).

Distributions of concentrations and sizes of airborne particles may also be measured (Cadle 1975). The measurements may be made with filtration, sedimentation, centrifugal, impaction, electrostatic-precipitation, or optical techniques. For high concentrations of large particles, filtration through fine mesh filters provides quick, inexpensive sampling. The filter may be weighed before and after sampling to provide concentrations expressed as weight per unit of volume of air. This method may be useful around diet-preparation areas where concentrations are high. In rearing areas, the nature and concentrations of particles such as insect scales require equipment capable of measuring very small particles at relatively low concentrations. Particle counters using light-scattering principles can count individual particles as small as $0.3\text{ }\mu\text{m}$, and some of these instruments can indicate numbers of particles in various size ranges (Cadle 1975).

This type of equipment was used to sample particles at the U.S. Agricultural Research Service's Western Cotton Research Laboratory, Phoenix, Ariz. (table 1). The egg-laying room contained moths of tobacco budworm, *Heliothis virescens* (Fabricius); pink bollworm, *Pectinophora gossypiella* (Saunders); beet armyworm, *Spodoptera exigua* (Hübner); and cabbage looper, *Tricoplusia ni* (Hübner). Air was sampled from inside pink bollworm cages because they were the smallest and most numerous moths in the room. Air was also sampled near the middle of the egg-laying room and outside the laboratory.

These measurements indicated that very small particles were present in the moth cages and that dry, 95%-efficiency filters could remove them. The particle concentration at the outlet of the filter in the moth room was below the instrument's detection threshold. Many particles in the moth room probably entered from outdoors; counts in the moth room varied with outdoor particle counts during the day.

Reducing Human Susceptibility to Insectary Contaminants

A person's susceptibility to an allergen or an irritant is difficult to determine. Also, different people exposed to similar environments respond differently. This variation may be due to variations in the rate of clearance from the lungs, the effects of cigarette smoking, existing pulmonary diseases, and genetic factors. Preemployment screening may keep susceptible people from being exposed, and annual allergy tests and tests of pulmonary function may be used to monitor workers to detect de-

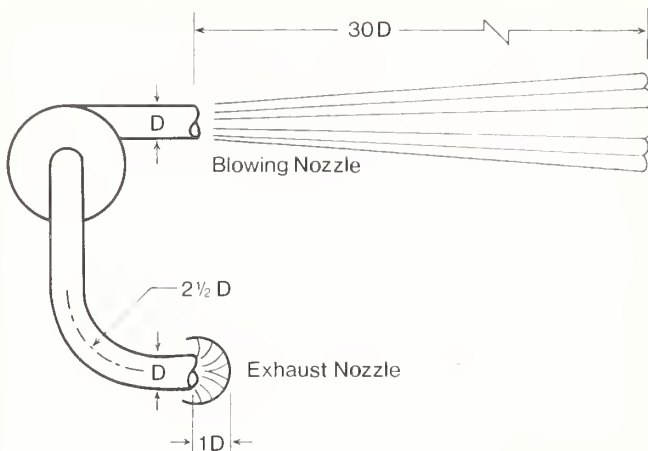


Figure 1.—Distances from blowing and exhausting nozzles where air velocity is less than 10% of velocity at nozzle opening. D=nozzle diameter.

veloping symptoms or susceptibility. Desensitization treatments benefit some individuals. These treatments and tests must be performed by qualified medical personnel. Low contamination levels reduce the probability of an individual acquiring a sensitivity to a specific substance. Engineering controls and proper work procedures can lower contamination levels.

Engineering Controls of Insectary Contamination

Engineering controls include mechanical equipment such as filters, ducts, containers, blowers, motors, and hoods to prevent contamination from reaching a worker. The selection, design, and use of such equipment are the most important steps in controlling respiratory hazards. Since particles that cause respiratory problems settle slowly, natural settling is not a solution. For example, $1\text{-}\mu\text{m}$ sized sawdust particles need 75 hours to settle 1.5 m (McDermott 1977). Small particles remain suspended in the air almost like gas molecules.

Applying controls near the source of contamination generally uses less energy, costs less, and works best. The use of air jets to move particles away from a work station usually worsens a problem because turbulence associated with the jet spreads the contaminants downstream and may even entrain more particles. Exhausting air from around the source removes contaminants so they may be filtered from the airstream or safely discharged. The air velocity should be at least 0.5 m/s at the point where contaminants are picked up or entrained in the exhaust airstream. Unfortunately, air velocity is less than 10% of



Figure 2.—Insect cages mounted on exhaust ducts to control moth scales. Air passes through cages and is filtered by cyclone and dry filters shown in figure 4.

nozzle velocity at a distance of 1 nozzle diameter from an opening (fig. 1). This effect mandates that exhaust openings be located close to the contaminant source and also makes overhead canopy hoods ineffective in collecting particles from workbenches.

Many insects have been collected with oral aspirators that inject contaminants directly into a worker's lungs. Oral aspiration should not be attempted without a filter capable of stopping 99% of particles $0.3 \mu\text{m}$ in diameter.

Enclosures

Enclosures surround the source of contaminants to prevent them from mixing with room air. There may be provisions for filtering air in the enclosure, filtering recirculated room air, or exhausting dirty air outdoors. Enclosures include reach-in cabinets, enclosed shelves, and special containers. Enclosures offer good protection as long as the access openings are closed. When they are open, turbulence from work procedures, room air-conditioners, or the transfer of material into or out of the enclosure can mix contaminated air with room air.

Insect cages do not provide protection unless their walls retain small particles or unless air is exhausted from the cage. Cages can be made with material permeable to moisture and vapor but able to block small particles (material such as fine knit cloth or perforated plastic). Exhausting air from screened cages removes particles before they mix with room air, does not restrict access to cages, and removes particles disturbed during feeding and egg collecting (fig. 2). The air velocity through the screened



Figure 3.—Cabinets for separating egg-laying cages of different strains of insects. Each cabinet has air circulation from right to left across each shelf and has 35%-efficiency filters to collect scales.

walls should be 0.5 m/s or greater so that insect movement inside the cage does not mix particles from the cage with room air. The cages can rest on ducts with an opening to each cage. Room air flows through the cage, through the ducts, and is filtered before being discharged into the room. Duct sizes should change at each branching point to maintain adequate airflow between cages. Since changing the duct size at each cage is not practical, the duct for each row of cages should be large enough so the end cage gets adequate ventilation. When several strains of one species need to be separated, egg-laying cages may have to be placed in separate cabinets (fig. 3).

Receiving hoods

Receiving hoods similar to those used above household stoves are generally not satisfactory for dust control because the air velocity at the work level is too low. These hoods are used when the work process creates enough heat that particles are carried into the hood as the hot air rises.

Capturing nozzles

Capturing nozzles normally consist of a duct with a fared opening. The fared opening (nozzle) is placed near the contamination source, and the exhaust air carries particles into the duct. Single nozzles are not satisfactory for most insectary work stations because the air velocity decreases so rapidly with distance from the nozzle. Adding more nozzles increases the capture zone but still may not provide protection if multiple insect cages or contamination sources are at the work station or are being transferred to the station.

Slotted ducts and perforation surfaces are also classed as capturing nozzles. Slotted ducts are normally located near the rear of the work station. Perforated surfaces on the rear of the work station or a perforated countertop provides the best airflow because clean air passes the worker's breathing zone, picks up particles in the work zone, and is exhausted.

More working space is generally available when perforated counters are used, because the exhaust ducts are below the table. Adding partitions to sides and tops of work stations creates a tunnel effect and improves the airflow near the worker's breathing zone. Supplying laminar-flow clean air above a work station and exhausting the air through a perforated counter provides the best protection.

An approximate method for determining quantities of air that must be exhausted to produce a desired capture velocity is presented in American Society of Heating, Refrigeration, and Air-Conditioning Engineers (1976, pp. 22.1-22.12). Air-duct design, loss coefficients, and recommended practices are described by Stomper (1979). Air-flow should be verified periodically with tracers such as smoke and air-velocity meters.

Filters

If dirty air cannot be exhausted outdoors, then it must be filtered before being recirculated in a room. The amount of air, dust-loading capacity, type of contaminant and smallest size of particle to be removed determine the type of filter needed. Dust collectors are used when the dust concentration is high and dust particles large, as in a large moth-holding room. They can collect large quantities of dust that would quickly load more efficient filters. Cyclone separators are the least costly dust collectors for medium-to-coarse granular dusts. They rely on centrifugal force to separate dust particles from the air-stream, are relatively small, and are inexpensive to maintain. High air velocities in the cyclone chamber require a high pressure drop across the collector; so operating costs and noise levels tend to be high. Few small particles are

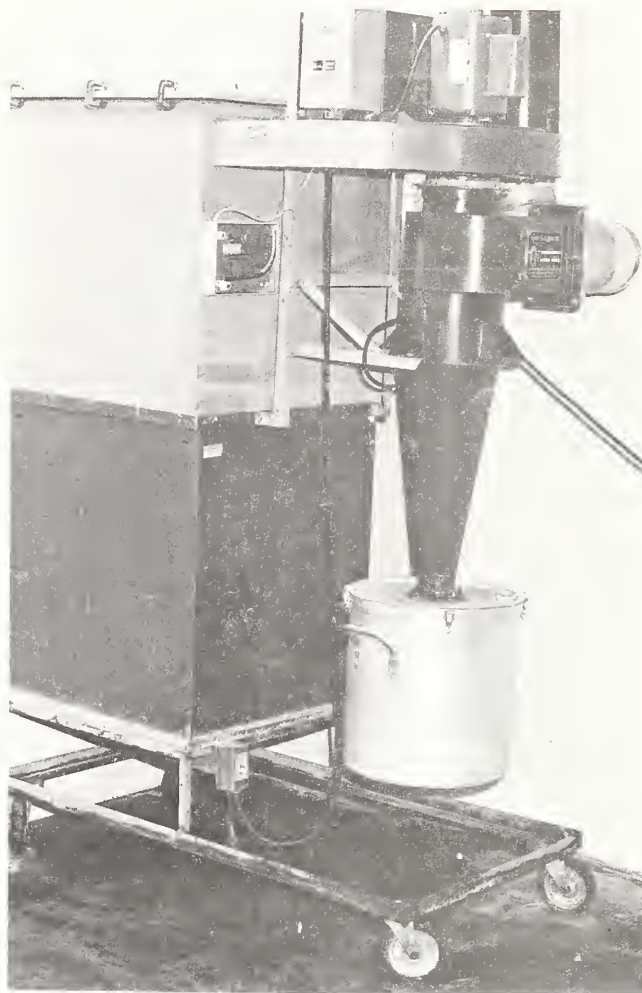


Figure 4.—Cyclone separator on right with 35%- and 95%-efficiency dry filters; charcoal filter on left. Used in moth room to filter scales collected from moth cages shown in figure 2.

removed by cyclone separators; therefore they are almost exclusively used upstream of more efficient filters (Stomper 1979). Cyclones in series use large amounts of energy because of the large pressure drop across each unit. Since a single cyclone removes the bulk of material, the remaining small particles can be removed with dry filters (fig. 4).

Fabric dust collectors use cloth tubes or envelopes. Particles larger than the fabric interstices are deposited by simple sieving action. A mat of dust forms on the fabric surface and improves the filtering efficiency. The collectors have built-in features to periodically remove accu-

mulated dust from the cloth surfaces. Common methods of dust removal include mechanical shaking, reverse-air collapse, and pulse jet. Airflow is stopped during cleaning. Dust is removed from the cloth and then removed from a drawer or hopper below the filter. These filters require less energy and are more efficient than cyclones, but initial expense and maintenance is greater. Filter cloth can be selected to remove 99% of particles 1.0 μm in diameter.

Dry filters are the broadest category of air filters for a variety of designs, sizes, and shapes. The most common filter medium is glass fiber because of its low cost and because the fiber diameter can be controlled during manufacture. Generally, the finer the diameter, the higher will be the air-cleaning efficiency. The fiberglass filters used in home furnaces are not efficient enough to remove most respiratory hazards. Hospital grade (95% efficiency) or industrial grade (99.97% efficiency) HEPA (high-efficiency particulate air) filters are recommended to adequately eliminate allergy, asthma, and pulmonary hazards (Zetterberg 1973). (Stomper 1979 discusses filter types and includes a guide to filter selection.)

Electronic filters of the type commonly sold for air-conditioning systems tend to lose efficiency as they remove dirt; they must be kept clean. They also generate ozone. They have a higher initial cost and are not as efficient over long periods of time as less expensive dry filters. Electronic filters maintain their initial low airflow resistance, even when loaded with dirt.

Activated charcoal filters remove many gaseous contaminants. They act as a catalyst to remove ozone and can remove most odors associated with insectaries. Charcoal filters are normally placed downstream of the last filter in the system. The types of gases removed and life expectancies of filters can be obtained from manufacturers' specifications.

Filter testing and rating is standardized industry wide with the American Society of Heating, Refrigeration, and Air-Conditioning Engineers test standard 52-76 (Stomper 1979). This standard describes tests for atmospheric dust spots and arrestance. But the tests do not indicate the removal efficiency for various sized particles. Some manufacturers provide information on filters that relates filter efficiency to particle sizes removed. HEPA filters are tested with a homogeneous fog of dioctyl phthalate (DOP), and particle concentrations upstream and downstream are determined to a high degree of accuracy with optical methods (Stomper 1979).

Respirators to Prevent Contamination of Insectary Personnel

OSHA mandates that engineering solutions be tried before respiratory equipment is used. But some work procedures, such as transferring insect cages to cleaning stations, may not have practical engineering solutions. Temporary jobs may involve transient exposure; and, if engineering solutions such as plastic bags, water baths, or special transfer carts are not possible for such jobs, then respirators may be necessary.

Respirators may be obtained for protection against dusts or gases. They should not be used in atmospheres that would pose an immediate health threat to a worker with no respirator. Only respirators that are certified effective for the type of contaminant encountered should be selected, and they must cover the mouth and nose. If the hazard is absorbed through or irritates the skin, then the skin should also be covered. The respirator should fit the individual user and not interfere with the work. Cloth or paper dust respirators are generally more comfortable to wear but provide less protection than cartridge respirators. Respirators are available that supply clean air to the worker via a hose from a remote supply or from a self-contained demand air supply.

An acceptable respiratory program should include the following elements:

1. Written standard operating procedures should be available for the selection and use of respirators.
2. Users should be instructed on proper use and limitations of respirators.
3. Certified respirators should be selected for the hazards involved.
4. The respirator must be properly fitted.
5. Respirators should be cleaned and disinfected as often as necessary to insure protection of the wearer.
6. Respirators must be used at all times when protection is required.
7. Respirators should be stored in a convenient, clean, and sanitary location.
8. Respirators used routinely should be inspected during cleaning.
9. Defective respirators should be replaced or repaired by experienced personnel before use.
10. There should be regular inspections and evaluations to determine the continued effectiveness of the respiratory protection program.

Cloth facelets, beards, glasses, or goggles that interfere with the sealing edges of the facepiece or valve action preclude the wearing of a respirator. Contact lenses should not be used by someone wearing a full facepiece or hood

respirator because the wearer would have to expose himself to recover or adjust them (Day 1979).

Conclusion

The unique nature of each insectary requires special hazard assessments and appropriate controls. Some insects tolerate various contaminants, so equipment can be designed to operate only when workers are present. Other insects require HEPA filters and equipment to protect them from bacterial, mold, and viral contamination. Such equipment provides some protection from respiratory hazard for insectary workers and may simplify the design of respiratory-protection equipment.

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General Requirements for Facilities That Mass-Rear Insects

By Jack G. Griffin¹

Introduction

A thorough knowledge of the unique combination of procedures, equipment, space, and environment requirements for rearing any particular insect is necessary for adequate planning of the rearing facility. Therefore, describing an ideal facility for rearing all insects is impossible. Most structures currently used for insect rearing were designed originally for other purposes (see, for example, several articles in Leppla and Ashley 1978) and modified later to house small laboratory colonies. So these facilities vary widely in type, floor plan, and construction method (see, for example, several articles in Leppla and Ashley 1978). And many facilities for large-scale rearing were planned and constructed with small-scale rearing systems as models and with general knowledge of the structural performance of similar buildings. Many of these facilities were modified almost immediately after becoming operational because some drawbacks that can be tolerated for rearing small colonies become serious in mass rearing, especially where contamination by micro-organisms is a problem. Unfortunately, not much research has been done on how facilities affect insect production. Despite these handicaps, we know enough about the general needs and problems of mass rearing insects to be able to describe certain common requirements for insect-rearing facilities. These include general space requirements, structure types, construction materials and methods, and environmental control and equipment.

Space Requirements for Rearing Facilities

Space requirements are not the same for all insects, but most need places for storage, diet preparation, egg production, egg or larval implantation, insect development, and adult emergence. For example, with the knowledge and experience gained in mass rearing boll weevils, *Anthonomus grandis grandis* Boheman, at Mississippi State University's Robert T. Gast Boll Weevil Rearing Facility at Mississippi State, Miss., I recommend that a facility for mass rearing this insect have 14 separate areas, each consisting of one or more rooms. This number is needed

to provide proper environments, sanitation and disease control, photoperiod, and security. Unfortunately, standards are not available for determining the space needed in insect-rearing facilities. The size of each area varies with use, insect to be reared, and production capacity. And, adequate room must be provided for equipment, work and storage space, and traffic lanes.

Structures for Rearing Facilities

Structures for rearing facilities must meet the building code of the location, provide the sanitary conditions and environments needed for the rearing operations, provide safety for workers, have adequate space, and be designed to permit efficient arrangement of each operation. Some types of buildings are better adapted and more economical than others for rearing insects. These structures may have metal or wood framework enclosed with various materials, or walls of cinder block and other masonry. The roof may be sloped or flat to accommodate air-conditioning and associated handling equipment. (Placing the air-conditioning equipment on the roof saves space, and the roof might be the most efficient location.) Modified mobile home units are also being used (J. Roberson, personal communication, and see, for example, Gantt et al. 1978), but these are not suitable for large, permanent facilities.

Considerations in planning rearing-facility structures include operation sequence, sanitation and disease control, production operations, workflow, and security requirements. These factors vary in importance with different insects. Sanitation, for example, is critical in boll weevil rearing and is given priority. Control of pathogens and other micro-organisms is probably one of the most important factors in producing large quantities of high-quality insects. Therefore, areas where contamination control is critical (rooms for handling sterile diet and rooms for insect development) should be separated from areas needing less isolation (egg-production colony, emergence room, and areas where spent diet or adults are handled). Also, some areas must be divided into separate rooms to further isolate the more critical contamination sources. Because of high levels of contaminants in some diet ingredients and their spread by air currents during processing, diet-preparation areas should be separated. Airlock vestibules reduce the amount of air that enters clean-rooms. Places for personnel to shower and dress may also be needed.

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The functions and sequences of each operation must be considered in arranging areas in a facility. For instance, diet sterilization and larval or adult diet preparation and dispensing should be located near each other. Areas should also be arranged to provide the most efficient flow patterns for materials and personnel. Good flow patterns reduce backtracking, save time, prevent traffic congestion, and prevent interference between locations. A central corridor adjoining all production areas and extending to the outside, if properly planned and arranged, can provide good traffic and workflow patterns. Or, all the production areas can be joined by a covered outside walkway. This approach is used at the R. T. Gast Rearing Facility. But items moved from one area to another would be exposed to outside contaminants, and insects would be more likely to escape. And the amount of travel between areas might be greater than with a central corridor. A combination of these two arrangements may be used to best advantage.

Construction Materials and Methods

Construction materials must provide permanency, cleanability, serviceability, economy, and safety. Usually, however, no one material rates high for all these criteria. For example, concrete flooring is the first choice for a permanent facility. If smoothly finished, it is slippery when wet; otherwise it is hard to clean and sanitize. It also produces dust particles when dry and can be damaged by some acids. So concrete finished rough or with an antiskid material applied to the surface can be used for floors that are wet much of the time and where sanitation is not a problem. Dressing and restroom floors can be plain concrete with a filler applied to the surface to reduce dust, or with a resilient floor cover added for better appearance. Shower-, equipment-, and maintenance-room floors that receive much wear, are frequently washed, require a surface that is not slippery when wet, and must provide a high degree of sanitation, should have a quarry-tile cover over a concrete base. A chemical-resistant grout should be used, and tile may be placed whole or broken to reduce its cost. These floors should also contain waterproof pans and drain outlets.

Materials are available for making a monolithic, seamless floor topping that can be used on concrete. These have not been tested widely in insect-rearing facilities; but one, an acrylic resin mixed with dry components and troweled on a concrete base in two layers, has delaminated from some floors at the R. T. Gast Rearing Facility. Another of these materials, applied on some floors at the U.S. Agricultural Research Service's Southern Field Crop Insect Management Laboratory, Stoneville, Miss., as a resinous mixture without any dry components, has also peeled from the base (C. W. Gantt, personal communication).

Some floors must be washed and rinsed with water and sanitizing solution to keep them clean. This operation is easier if floors slope to drains. P-traps should be used to prevent sewage gas from entering the rooms, and trap boxes are necessary to stop particles from clogging the lines. Floors should be insulated to prevent surface condensation in rooms that are maintained at warm temperatures and high relative humidities during winter. Cold floors also contribute to undesirable stratification of air temperature.

Cinder blocks have been satisfactory as interior walls in the R. T. Gast Rearing Facility. Originally, their surfaces were rough and porous, but three coats of epoxy enamel paint gave a smooth and durable finish. These block walls withstand the occasional physical abuse that inevitably occurs where equipment is moved. Plywood or Masonite sheets with a plastic layer laminated on one side also provide a surface that is tough and easy to clean. They are used in food-processing areas but are suitable elsewhere. Gypsum board or plaster painted with epoxy enamel has served satisfactorily in areas where the walls are not subjected to excessive moisture or wear. Surfaces of plaster and gypsum board should be finished smoothly for easy cleaning and sanitizing. Walls for shower stalls and maintenance rooms usually have ceramic tile on a masonry base. Preferably, restroom walls should have 1.2-m-high ceramic tile wainscots, or the entire wall may be covered with tile, tempered Masonite, or exterior plywood boards with a layer of plastic laminated to their interior surfaces. Metal walls are suitable in storage rooms unless they are exposed to corrosive materials. Stainless steel can be used in some areas, but it is not recommended. Joints between the floor, walls, and ceiling must be without crevices, so sometimes they are joined by a masonry base. The base should be recessed flush with the wall surface to avoid formation of a ledge. Metal beads should not be used because they will corrode. Holes for utility lines that enter the wall cavity should be sealed to prevent entrance of dust and microorganisms. Walls should be a minimum of 2.4 m high, and their exterior sides should be insulated to reduce heat transfer, room-temperature stratification, and condensation.

Gypsum board or plaster coated with epoxy paint will usually suffice for ceilings in areas not subjected to high moisture or overhead leaks. Otherwise, exterior-grade plywood or tempered Masonite with a laminated plastic covering should be used. A good, tight fit, or a specially fabricated metal connector strip, is needed with this type of construction. Also, any openings for light fixtures or utility lines should be sealed. Ceilings should be insulated to facilitate temperature control in heated and cooled rooms.

Windows are usually used for light and esthetics rather than for ventilation. They should be kept to a minimum and be nonopening, stationary panels with frames made of heavy-duty metal. Windows in heated or cooled rooms should be insulated. All hinged doors should be hollow core with a glass panel near eye level. Both frames and doors should be made of heavy-duty metal. Outside doors should have sturdy door closers and weatherstrip seals. Doors between rooms that are maintained at different levels of cleanliness, air temperature, and relative humidity should have automatic bottom seals. Oversized outside doors are essential for transferring equipment. Interior doors should also be wide enough to accommodate equipment, at least 1.1 by 2 m. For restrooms and showers, 0.9-m-wide doors are adequate.

Environmental Control and Equipment

Some rearing facilities use individual electric or gas heaters and window air-conditioner units for each room. But this approach is neither the most desirable nor most economical for large operations. Central heating and cooling are preferable. Typically, hot water is the source of heat, and cold water is used for cooling. The water flows through coils that are positioned in the ducts of the air-handling system; a blower circulates the air. The water is heated with steam or some type of fuel or is cooled to about 8° C with a refrigeration-liquid chiller. Controls are available for modulating the flow of water through the coils to maintain the proper air temperature. In egg-production, insect-development, and adult-emergence rooms, the differential of the controlling device should not be more than 2° C and about 4% relative humidity. Individual air-handling systems can be used to provide different temperature, relative humidity, and sanitation levels in the facility. For example, 12 units are installed in the R. T. Gast Rearing Facility. Fresh air is brought in and conditioned before being mixed with the recirculated air. An exhaust system is essential, and return outlets must be located to provide for a uniform air movement, temperature, and relative humidity throughout the rooms.

Cooling coils may be used to partially dehumidify. But, since the vapor pressure of air passing the cooling coils is not reduced very much, supplementary dehumidifying equipment is usually necessary. The heating coil is placed downwind from the cooling coils when they are used to dehumidify the air. The most desirable way to add moisture is to use humidifiers that inject steam into the duct. An override humidistat should be located in the air duct on the room side of the humidifier to prevent occurrence of too much moisture. Fogging nozzles and rotating watering wheels or drums are less desirable than a steam injector because they often cause excessive fungal and algal growth. Also, it is important to insulate the outside

rather than the inside of the duct. The system's control sensors for air temperature and relative humidity should be located to sense average conditions of the air in a room.

Light should furnish proper illumination for work to satisfy requirements of the insects, either from natural light through windows, from artificial light, or from both. Light fixtures should meet their use requirements (be dust, moisture, and insect proof). Fluorescent lighting is more efficient than incandescent in output per unit of electrical energy. For insects that require both light and darkness during each 24-hour period, lights can be controlled by a time switch. Care must be given to the intensity and distribution of light if the insects are phototrophic. A natural or artificial high-intensity source can cause some insects, such as the boll weevil, to crowd toward the light. Weevils spread more uniformly over the diet pellets when exposed to uniform, indirect lighting or to total darkness. But high-intensity artificial light is sometimes helpful in insect rearing. For example, it is used to attract weevils from the rearing medium after they have developed to the adult stage.

Air movement is critical in production rooms. For example, too much air movement around rearing trays in a boll weevil development room dries the rearing medium too fast and causes nonuniform development and lower yields. Similar problems occur in weevil oviposition and emergence rooms. In rooms where sanitation is critical, air supply outlets from the conditioning system should be covered with hospital grade (minimum) HEPA (high-efficiency particulate air) filters. The return-air opening in all rooms must have a standard furnace filter. The added static pressure caused by the HEPA filters must be considered in selecting blowers for the air-handling system. Some HEPA filters require 2.54-cm wg static pressure to maintain their rated airflow. Laminar-flow cleanrooms should be used for handling diets and other materials that must remain free of microbial contamination. Also, the rearing facility should be kept as clean as possible by good housekeeping practices to minimize dependence on air filters. Some insects shed scales and other particles that can be harmful or at least annoying to workers (Mangum et al. 1969). If such insects are present, air must be recycled through filters often enough to keep it clean.

Airlock, passthrough cabinets may be used for passing materials between rooms in areas where traffic is restricted for sanitation purposes. These passthroughs may contain ultraviolet lamps to help reduce transfer of airborne micro-organisms. Proper types and amounts of weather-stripping and sealing materials must be installed on all doors. Rearing facilities often require autoclaves and ethylene oxide fumigation chambers. These units can

have a door on each end and can be used between rooms as passthroughs. Formaldehyde gas fumigation chambers used in the R. T. Gast Rearing Facility are gastight and exhausted through the roof. Each fumigating chamber is controlled automatically with a timer and an actuator on the dampers of the exhaust and fresh-air outlets.

A central vacuum system for wet-dry cleaning of floors would be helpful, especially for insects that require a high degree of sanitation. The lines for the vacuum system should be made of a noncorrosive material. Compressed air is needed for some operations and equipment. There should be a backup compressor and motor for the system. An air dryer and filter should be used in the lines.

Several details are important to maintaining the necessary environment and equipment in a rearing facility. Control of the environment is lost if central heating or cooling breaks down; a backup unit is recommended for each of these. Some rearing operations require pure water; so a distilling unit, holding tank, and distribution lines have to be installed. Sinks and benches should be made of heavy-duty stainless steel with rounded edges for cleaning and safety; drainboards may also be useful. Smaller pieces of equipment and materials should be stored on open racks or tables. Refrigerators and freezers should be made of stainless steel to withstand the clean-

ing and sanitizing agents. The compressors for walk-in units located in dusty places (such as those units used to store or prepare diet ingredients) should be installed in an adjacent clean area. Fire extinguishers of proper type should be located conveniently in the facility. Safety lights that activate automatically should be provided. An internal communication system that can be used without any manual operation is important for coordinating work performed in separated areas (such as diet sterilization and placement); workers should have outside communication equipment when they are restricted to a certain area of the building.

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Automation in Insect Rearing

By E. A. Harrell¹ and C. W. Gantt²

Insect-rearing procedures are slowly but surely being automated, or mechanized, because demands for laboratory-reared insects are growing and costs are increasing rapidly. "Automation" is the substitution of mechanical or electrical devices for human labor; "mechanization" is basically the same process, and we will use the two terms interchangeably here. The decision to automate, in most instances, results from attempts to complete a difficult task as quickly and cheaply as possible, not necessarily to replace labor as is sometimes thought. In insect rearing, automation may increase speed, reduce labor, eliminate human error, make operations uniform, improve insect quality, and increase the number of insects reared.

Many industries, especially electronics, have made tremendous progress in automation. Recently, the electronics industry has developed sophisticated solid-state controls, chips, and microprocessors. Solid-state controls to regulate temperature are becoming common in insect rearing. Microprocessors are not used so commonly, but they will be in the near future.

As late as the midsixties, most insects reared in small numbers were laboratory-reared on plant parts for food. With the advent of laboratory diets (meridic media), many techniques and apparatuses were developed for their preparation and handling. As the diets, equipment, and techniques became more sophisticated, usually the number of insects reared increased, and the cost per insect decreased.

For instance, an output of corn earworm, *Heliothis zea* (Boddie), was improved from about 500 to 2,000 per day by modifying and adapting a food-packaging machine to dispense a diet (Burton et al. 1966). This machine positioned 30-ml cups and metered diet into them in a continuous operation. To further use the potential of the packaging machine, equipment was designed and built to place a predetermined number of eggs in a cavity (pickout) on the cap (Harrell et al. 1970) and restack the caps mechanically so that they could be placed on the cups by the packaging machine. This innovation increased the corn earworm output to about 30,000 per day.

With the increased rearing capability, collecting pupae by hand from rearing cups became almost impossible. So researchers designed and built a mechanical pupae collector with a capacity of about 5,000 cups per hour (Harrell et al. 1969). It required one operator and increased his output tenfold. The mechanical pupae collector was 90% efficient, which was adequate but not as good as manual collection. Up to this point in the mechanization of corn earworm rearing, production was almost doubled and the cost per insect reared reduced by about half. Corn earworm rearing was further automated by adapting a 381-cm-long by 64-cm-wide by 127-cm-high inline form-fill-seal machine (Sparks and Harrell 1976). Powered electrically and pneumatically, the machine forms plastic into a continuous web of rearing cells, heat-seals a cover over them, and shears the web into desired lengths. The machine has been synchronized to accomplish the simultaneous operations of forming, sealing, and shearing at a maximum of 17 strokes per minute. A diet- and egg-filling station was designed, built, and installed between the forming and sealing heads to make a continuously automated process with a capacity of about 160,000 corn earworm rearing cells per 7-hour run (Harrell et al. 1973; Harrell, Sparks, Perkins, and Hare 1974). A diet proportioning, mixing, and sterilizing system was semiautomated to supply the form-fill-seal machine (Harrell, Sparks, Hare, and Perkins 1974). Also, a collector was designed and built to remove pupae from this type of cell. Corn earworm diet is dispensed into individual rearing cells while it is hot (42° C) and fluid. Eggs are placed in small, adjacent connecting cells where they are protected from the hot diet and microbial inhibitors. Our unpublished research has shown that these inhibitors injure eggs of corn earworm; fall armyworm, *Spodoptera frugiperda* (J. E. Smith); cabbage looper, *Trichoplusia ni* (Hübner); and boll weevil, *Anthonomus grandis grandis* Boheman, when they are in contact with the diet before it solidifies. After the eggs hatch, small larvae move through the connecting tunnel into the diet-filled cells and feed. This connecting-cell method works well for insects with mobile larvae and eggs that can be separated but not for insects, such as the boll weevil, that are almost immobile in the larval stage. Several eggs (three to four) are used per cell, but, because corn earworm larvae are cannibalistic, usually only one survives per cell.

Perhaps the most widely known and successful effort in automating insect rearing is that for the program rearing the screwworm, *Cochliomyia hominivorax* (Coquerel). The original program, begun in the midfifties and improved until the early sixties, produced enough flies to eradicate the screwworm from the southwestern United States (for

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details about the rearing procedures, see Goodenough and Brown 1976).

Between 1975 and 1978, boll weevil rearing received an extraordinary amount of engineering effort for automation. The petri dishes that had served as rearing containers, and the associated manual manipulations, were replaced by plastic trays that were formed, filled, and sealed in a continuous process (Harrell et al. 1977). The equipment was similar to that used for rearing the corn earworm except that the auxiliary devices and rearing trays were designed specifically for the boll weevil. Also, the diet was rapidly cooled to form a surface skin before the eggs were added. The eggs were sprayed on the diet in a uniform pattern and covered with a granular material that absorbed the carrier liquid and provided support for the young larvae. Equipment currently developed has the capacity to process enough trays to yield about 1 million weevils per hour (Harrell et al. 1980).

Progress has also been made in mechanizing processes associated with the weevil brood colony. Adult diet is prepared, sterilized, made into pellets, and covered with wax semiautomatically (Griffin and Lindig 1974). In this process, diet is pumped through a sterilizer and into the pellet-making machine where it is cooled, solidified, pushed out of pipes, and sheared into the desired lengths. The pellets then pass through a vat of hot wax where they are coated. These wax-coated pellets provide food and oviposition sites for the boll weevil. After the eggs are laid in the pellets, the wax is separated, recovered, and returned for recycling. The eggs are then mechanically removed from the pellets (Griffin and Lindig 1977).

Some of the time-consuming and tiresome jobs of rearing systems such as implanting eggs in cells by hand, handling the rearing trays on a conveyor, and metering specified numbers of eggs into the cells are being automated. A system has been developed for handling eggs without hand labor (McWilliams et al. 1980). It uses oviposition cages that provide for adult emergence, feeding, and oviposition. The cages are housed in a chamber that confines and collects moth scales, which are a potential human health hazard.

Many other insect-rearing procedures have been partly automated. For example, Gantt et al. (1976) reported that a valveless piston-type pump was developed and used in a project for suppressing the sugarcane borer, *Diatraea saccharalis* (Fabricius). The pump dispensed maggots collected from a tachinid *Lixophaga diatraeae* (Townsend) into host-rearing containers (plastic cups, 22.5–30 ml). This development increased production 17 times over previous manual methods.

A procedure that needs to be automated is the stacking

of trays from the boll weevil form-fill-seal machine. Presently, two or three workers are required to stack the trays when they are processed at speeds of 30 to 35 trays per minute. The food industry has the technology available to mechanically fill a room with racks and remove processed packages from a machine and stack them on the racks. Perhaps this system can be adapted to the needs of boll weevil rearing.

The environment is very important to the survival of an insect and its progeny; therefore the insectary must be monitored and controlled within specified limits. Perhaps the most critical areas are the rooms for oviposition, processing of rearing containers, and larval incubation. Computer-operated electronic controls are available, but the degree of control is no better than the sensors. Unfortunately, however, progress on sensor development has not been as rapid as that on controllers. Sensors for dry-bulb measurements such as thermocouples, thermistors, resistance bulbs, and thermostats are dependable and accurate. Commercially available proportioning controllers will maintain dry-bulb temperatures very precisely when used with resistant-type electrical heaters. Control of relative humidity is more difficult than control of temperature; it is usually obtained by measuring relative humidity or dewpoint temperatures. Some of the more precise sensors require considerable attention and maintenance. Also, their location must be selected carefully to satisfy their operational characteristics. Perhaps the best method for monitoring and controlling the environment in a room is to saturate air at a known temperature with water vapor and then change the air temperature to obtain the desired relative humidity. Steam can be added to increase it, or it can be lowered with desiccants or refrigeration equipment.

Automation will probably pay for itself. For example, mechanization of corn earworm rearing (diet preparation, tray processing, pupal collection, etc.) at the U.S. Department of Agriculture's Southern Grain Insects Research Laboratory at Tifton, Ga., reduced costs by \$8.91/1,000 (late 1970's). And replacement of petri dishes with trays for rearing the boll weevil saved about \$75 per hour. Additional savings and increased capacity were achieved by mechanizing the operations of filling the trays with diet, adding the sand, and applying the cover. In these cases, automation was essential because the programs required large number of insects daily.

Although those of us involved in rearing insects have made tremendous progress in automating our procedures, we have not kept pace with industry, nor have we adopted all appropriate industrial technology. Unfortunately, no one has capitalized on opportunities to design and build an automated rearing facility. Instead, automation has been piecemeal: the few engineers active-

ly researching this capability have concentrated on isolated problems rather than on the design of complete systems. This approach can cause imbalances and bottlenecks in the sequence of operations; some areas may not have the capacity to handle the additional workload created by mechanizing another. When mechanizing some part of a rearing system, one should account for the complete rearing regime to achieve a smoothly operating and efficient system for mass producing quality insects.

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Materials Handling in Insect Rearing

By J. L. Goodenough¹

Introduction

Insect rearing involves many and varied materials-handling processes. These range from implanting eggs in small dishes to receiving semitrailer loads of diet. This paper considers materials handling as processes; these are described by action verbs such as "move," "combine," "mix," and "separate." Such actions are involved in the warehousing of materials in diet preparation, and in the rearing, packaging, and distribution of insects. Rearing is divided into egg handling, larval rearing, pupal handling, maintenance of adult colonies, egg production, and associated operations. Examples are included here to illustrate present technology (developed mainly by engineers and biologists working jointly) and to support recommendations for further work. The reference to physical and mechanical properties of both diet and insects and rheological properties of diet materials should increase biologists' understanding of terms used by engineers to describe properties of materials. Data on these properties, which are essential to food engineers, barely exist in the field of insect rearing.

Warehousing of Materials

Is the warehouse nothing more than that leftover space where we pile junk, or is it intimately related to packaging, maintenance, and procurement? Careful planning is required to provide an adequate facility that will include the necessary space; proper environmental control; and procedures needed for receiving, storing, and retrieving all required materials. And planning must account for the multitude of ways materials are packaged. For storage facilities and procurement sections to be properly coordinated, adequate records and inventory control must be provided. Records and control must insure that the procurement section is not surprised by last-minute orders and that the warehouse is not caught without space or personnel to receive a shipment.

Emphasis on warehousing is evident in reports of large-scale insect-rearing systems, especially the need to store diet materials with minimal loss of quality. Harrell and Griffin (1981) described special warehousing facilities for storage of the dried diet products, corncob grits, and

sand needed for mass rearing the boll weevil, *Anthonomus grandis grandis* Boheman. They recommend a building located separately from the main rearing facility to reduce transfer of dust particles and micro-organisms into the rearing area and to discourage personnel from moving between storage and rearing areas without following proper sanitation procedures. Two cool, dry rooms would be necessary to store the various diet materials; one of these would have temperature and humidity control. A gasoline-engine-driven forklift would be used to transport diet materials from storage to preparation areas. Bell et al. (1981) reported that the expanded gypsy moth, *Lymantria dispar* (Linnaeus), rearing and virus-production system included a cool room for storage of diet ingredients in bulk and a reach-in commercial freezer for storage of vitamins in bulk and for holding pre-weighed batches of dry diet ingredients. The screwworm, *Cochliomyia hominivorax* (Coquerel), rearing program (Goodenough and Brown 1976) had a central warehouse (remote from the mass-rearing facility) for receiving and storing dried diet products, packaging materials, and miscellaneous tools, repair parts, and supplies in bulk and for dispensing items to the many program elements.

Handling Materials in Diet Preparation

In the study of materials handling in insect rearing, the greatest effort has been spent in trying to simplify and streamline diet preparation. The materials-handling actions researched most often include measurement, sterilization, mixing, conveying, and dispensing. Most processes include heating and cooling. Some diets are pelletized and some are encapsulated. Harrell and Griffin (1981) reported that grinding and sifting is necessary in preparing boll weevil diets. Some diets are held in cold storage after preparation until used (Barnes 1976, Baumhover et al. 1977).

Various types of controls are used to measure or meter diet and to adjust diet temperature. Griffin and Lindig (1974) operated a manifold and controlled valves by hand in making diet pellets for boll weevils. This operation was later automated with electric and pneumatic control of ball valves (Griffin 1979a). An adjustable timer and solenoid valve have been used to control the rate of diet dispensed with a multiple-species diet dispenser (Gantt and King 1981). Harrell et al. (1974) metered dry diet materials with a vibrator-agitator. Gantt and King (1981) designed and built a special check valve to control dripping of the applicator nozzle between filling of diet cups. Temperature control of diet has been reported necessary

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by Baumhover et al. (1977), Harrell et al. (1977), and Gantt and King (1981). Bell et al. (1981) noted the advisability of controlling the entire diet-preparation procedure to help maintain uniformity among batches.

Many materials are routinely sterilized during diet preparation. For rearing the gypsy moth, Bell et al. (1981) boiled all diet ingredients during processing to inactivate autolytic enzymes and microbial contaminants that promote food spoilage. Diet has been sterilized in production of the boll weevil (Harrell et al. 1977) as have been granular material (Griffin 1978) and diet materials, corncob grits, and sand (Harrell and Griffin 1981). Griffin et al. (1974) reported flash sterilization and automatic temperature control. Also, Griffin and Lindig (1974) reported sterilization of diet equipment for producing boll weevil food pellets, and Griffin (1979b) reported sterilizing the cooling tunnel used in producing boll weevil diet.

An element of diet preparation common to nearly all insect-rearing programs is mixing. Bell et al. (1981) blended gelling agents for gypsy moth diet; then the dry mixture was blended into the gel with a steam-jacketed kettle mixer. Small experimental batches were prepared in a blender. Barnes (1976) reported that in a program for rearing the Natal fruit fly, *Pterandrus rosa* (Kuschel), diet ingredients were mixed first; then water was added, and the aggregate was mixed well. Mixing of dry diet materials and then adding water is a procedure also used by Harrell et al. (1974) for corn earworm, *Heliothis zea* (Boddie), diet and by Harrell and Griffin (1981) in preparing boll weevil diet. In contrast, Goodenough and Brown (1976) reported that, in preparation of screwworm diet, dry products were mixed into water by suction (via a modified venturi section). And, as the water-nutrient mixture was recirculated from one of two mix tanks (2,300-liter capacity each) through the top of an inverted T-section, measured amounts of dried nutrient products were vacuumed into the mixture through the stem of the T; this method provided uniform mixing of the dry ingredients. Another mixing operation is required in some programs to incorporate preservatives.

Large volumes of diet are usually moved by pumping after they have been mixed. Griffin and Lindig (1974) and Harrell and Griffin (1981) reported boll weevil diet being pumped into a pelletizing machine and also from a sterilizer to the holding tank of a filler for larval rearing trays. Harrell et al. (1974) used cool sterile diet pumped into a diet filler for rearing the corn earworm. Gantt and King (1981) pumped high-temperature insect diet into rearing cups with a multispecies diet dispenser. Goodenough and Brown (1976) reported that liquid diet for screwworms was pumped from mixing tanks through a recirculating system and sprayed onto larvae-rearing

vats; continuous agitation in the recirculating system kept the diet materials in suspension.

The final stage in diet preparation for at least two species is pelletization. Griffin and Lindig (1974) reported mechanization of this process for boll weevil diet. Griffin (1979a) reported an automatic control and manifold for producing the food pellets. Also, to provide an oviposition substrate, Griffin and Lindig (1974) reported coating adult boll weevil diet with wax. A diet formulation for rearing the common green lacewing, *Chrysopa carnea* Stephens, was reported by Martin et al. (1978) who encapsulated the diet in a shell of four waxes.

Egg Handling

Collecting eggs

There are nearly as many methods of collecting eggs as there are systems to produce them. Griffin and Lindig (1977) and Harrell and Griffin (1981) reported harvesting boll weevil eggs after adult weevils had oviposited in food pellets. Egg-implanted pellets were vibrated into a "cracker," which broke the wax coating either with a rotary chopper or by forcing pellets between two rollers. After the wax was skimmed or floated off, water, diet particles, and eggs were pumped into an egg-diet separator, where the eggs were recovered by a series of wire-mesh screens and water rinses and cleaned with saturated NaCl-brine solution. Harrell and Griffin (1981) included a system for recycling the reclaimed wax. Bell et al. (1981) reported egg masses of the gypsy moth being scraped from removable paper liners placed inside heavy-duty paper oviposition containers. Morrison and Hoffman (1976) collected Angoumois grain moth eggs by vigorously brushing them from nylon screen. Reeves (1975) reported common green lacewing eggs being collected from paper cage liners; a cage-liner cutter and two mobile racks held cage liners after diet application until installation in cages; the racks were also used to hold liners removed from cages until eggs could be collected, thus significantly reducing space and labor required to handle them. Screwworm eggs were scraped by hand from wooden oviposition frames with kitchen spatulas (Goodenough and Brown 1976). Barnes (1976) reported most eggs of the Natal fruit fly laid through filter paper being easily soaked off in a beaker of water and collected by filtration. Tobacco hornworm eggs laid on artificial leaves can be easily removed by hand (Baumhover et al. 1977).

Harvest of Angoumois grain moth eggs included removing insect scales mixed with eggs by using a screen shaker (Reeves 1975); scales were eliminated from the air in oviposition and egg-collection rooms with an air-filter system; hoods were placed over the cage-holding area and

egg-collection table; a high-pressure centrifugal fan pulled air laden with insect scales from the hoods through ducts and through a single-stage, impingement-type wet-gas scrubber, that collected 99% of the scales. Mitchell et al. (1975) cleaned fruit fly (*Tephritidae* spp.) egg-production cabinets with steam because, in addition to cleaning, it killed any remaining adults and prevented their escape.

Surface sterilization of eggs

Egg preparation for implanting on diet material may include surface sterilization. For example, Bell et al. (1981) reported using a 10% Formalin (formaldehyde) solution to remove virus from gypsy moth eggs, and Harrell and Griffin (1981) found that boll weevil eggs have to be surface-sterilized. Baumhover et al. (1977) reported tobacco hornworm eggs being surface-sterilized, rinsed, and dried.

Measuring eggs

Eggs are usually measured in some way. For example, measurement of tobacco hornworm eggs before implanting was reported by Baumhover et al. (1977) to be by weight or volume. Goodenough and Brown (1976) reported screwworm eggs being weighed on a pan balance, and Mitchell et al. (1965) reported measuring fruit fly (several species of *Tephritidae*) eggs volumetrically in water.

Dispensing eggs

Sparks and Harrell (1976) reported the first mechanized dispensing of corn earworm eggs; casein glue was dispensed on paper bottle caps, and then six to eight eggs were placed on the glue. The machine then inverted and stacked the caps. In a later development, a vibratory feeder was used to add eggs to cells formed by a form-fill-seal machine. Bell et al. (1981) reported gypsy moth eggs being placed into petri dishes. Barnes (1976) placed Natal fruit fly eggs on artificial diet medium just before they hatched. Goodenough and Brown (1976) reported 7-9 lots of screwworm eggs being placed onto damp paper towel in petri dishes. And boll weevil eggs were sprayed onto diet as part of a system using a form-fill-seal machine (Griffin 1978, Harrell and Griffin 1981); the machine first formed plastic trays and dispensed diet into them; then, measured amounts of eggs were sprayed onto the diet; the eggs were uniformly suspended by maintaining the proper specific gravity of a starch solution (Gast 1966, Griffin 1978); after eggs were dispensed, the diet was covered with a granular material. This unit appears to be the most automated system yet developed for dispensing rearing medium and implanting eggs on it.

Incubating or storing eggs

Egg-handling procedures may include only incubation. Barnes (1976) reported Natal fruit fly eggs being held in a saturated atmosphere until just before hatching. Then, newly hatched larvae were placed on artificial medium. Screwworm eggs hatch after being held about 12 hours in an incubator. Excess production may be chilled and held for short periods (Goodenough and Brown 1976). The longest period for chilling and holding egg masses that I have found is that of 5-6 months in rearing gypsy moths (Bell et al. 1981).

Handling Materials for Rearing Larvae

Handling larvae

Goodenough and Brown (1976) reported that in the screwworm rearing program, soon after eggs hatched, the larvae were transferred from petri dishes to small pans containing starting medium. After 24 hours, these larvae were transferred from the small starting pans to large pans and later to 1.2- by 1.8-m vats. The rearing vats were stacked five per rack onto racks suspended from a monorail. Developing screwworms were moved progressively through rearing, pupation, and pupae holding areas on the monorail system. In the gypsy moth rearing program, newly hatched larvae were placed into 180-ml diet cups by brushing (Bell et al. 1981) and then transferred to the main rearing room. After 21 days, larvae were transferred to 500-ml cups that contained fresh diet (see below). In contrast, Harrell et al. (1974) and Sparks and Harrell (1976) reported that food was already provided before corn earworm eggs were metered into rearing cups. To facilitate transporting the rearing medium after eggs were added, manual stacking was required for corn earworm rearing cups and trays (Sparks and Harrell 1976) and for boll weevil rearing trays (Harrell et al. 1977; Harrell and Griffin 1981).

Feeding larvae

Supplemental feeding is required in rearing screwworm larvae. Goodenough and Brown (1976) reported that liquid screwworm diet was added to larval rearing vats at 4-hour intervals. The liquid diet was added by feeder lines of a system that continuously circulated the diet. Several researchers have reported adding diet only at the initial filling of rearing containers (Baumhover et al. 1977, in rearing tobacco hornworms; Barnes 1976, in rearing Natal fruit fly; Sparks and Harrell 1976, in rearing *Heliothis* spp.; and Harrell et al. 1977 and Griffin et al. 1979, in rearing boll weevils). Because of high amount of labor required and difficulty in applying conventional

automated rearing technology, Bell et al. (1981) desired not to change diet during the long feeding period of gypsy moth larvae; but, because no available diet was nutritionally stable the required length of time, insects were usually transferred to fresh diet after 21 days. Although Bell et al. (1981) had not completed their tests, they did find that doubling the vitamin mix and boiling all diet ingredients during processing resulted in successful rearing without transfer of larvae to fresh diet.

Temperature control in rearing larvae

In some rearing systems, as larvae mature, the increasing amount of metabolic heat produced may cause problems that require special materials-handling procedures. Tanaka et al. (1972a) found that metabolic heat had raised the rearing-vat temperature enough to reduce the rate of larval growth in Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann); they corrected the problem by moving the trays of larvae into a cooler rearing room, which resulted in increased yields. Or they could add water to rearing trays if the cooling units failed. Goodenough and Brown (1976) reported that rearing-medium temperature was controlled by disconnecting vat heaters when screwworm larvae were large enough to produce an appreciable amount of metabolic heat.

Harvesting larvae

A wide variety of materials-handling procedures have been used to harvest larvae. Screwworm larvae were harvested by adding water to the center of the rearing vats, which induced the mature larvae to crawl off (Goodenough and Brown 1976). These larvae fell into water flowing in a gutter, were elevated by jet action (injector), and then were separated from the water with a shaker. Larvae moved across the vibrating rack of the shaker and fell into a bin. They were then scooped onto wire-mesh screen placed over pupation trays. The larvae crawled through the screen into a sawdust pupation medium, leaving trash on the screen. Trays of larvae were transferred to the pupation room via monorail. Barnes (1976) used a combination of inverting rearing trays and low temperature to induce Natal fruit fly larvae to crawl out of the medium through gauze and fall into a box of damp sand. Baumhover et al. (1977) reported that, because of their cannibalistic behavior, tobacco hornworm larvae had to be removed from the rearing trays and placed in individual pupation cells.

Sanitation in rearing larvae

Sanitation was reported as critical in corn earworm rearing by Sparks and Harrell (1976) and has been imperative in increasing production of gypsy moths and reducing the necessity to replace larval diet (Bell et al. 1981). General

sanitation operations include cleaning rearing vats (Goodenough and Brown 1976), cleaning floors and walls, fumigating the rackveyor (Harrell and Griffin 1981), and cleaning media cabinets with steam (Mitchell et al. 1965). In tobacco hornworm rearing (Baumhover et al. 1977), all equipment was routinely soaked overnight or longer in a 0.5% Chlorox (0.026% sodium hypochlorite) solution for cleaning and sterilization; the stored equipment was soaked again just before use; any units that had unhealthy larvae or active colonies of bacteria or fungi growing on the diet or fecal pellets were removed from the rearing area. Sengupta and Yusuf (1974) reported silkworm, *Bombyx mori* (Linnaeus), rearing beds being cleaned once a day and dead and diseased worms being removed. Sanitation to provide suitable holding conditions sometimes includes filtering of air. Incoming air is filtered to remove bacteria and fungi before passing over boll weevil rearing trays (Griffin 1979b). Filtering is done within the airflow system used in the room for rearing gypsy moth larvae to remove insect hairs and scales and to reduce the incidence of airborne microbial contaminants (Bell et al. 1981).

Materials Handling for Pupation

Handling pupae

Generally, after trays of pupae are loaded into holding racks, they are transported into holding rooms and held for further development. Some systems require that pupae be separated from holding medium before being irradiated, packaged, or returned to the adult colony. Young et al. (1980) separated corn earworm pupae from unused diet, spent diet, and frass residue by floating the insects in water at negative pressures of 380- to 610-mm Hg. And Gross and Young (1978) reported that dead corn earworm pupae and pupae that produce deformed adults float without vacuum. Tobacco hornworm pupae were held in pupation cells for 7 days and then transferred to screen trays in single layers, or prepupae were placed in a moist soil substitute (vermiculite and sand, 1 : 1) and later sifted (Baumhover et al. 1977).

In the screwworm rearing program, after 12-20 hours in the pupation room, trays containing pupae, larvae, and sawdust were emptied onto a shaker-separator, which separated sawdust from pupae and larvae (Baumhover et al. 1966, Goodenough and Brown 1976). Sawdust was recycled until it became too dirty to use. Larvae and pupae were conveyed on a continuous belt through a tunnel of fluorescent lamps, which caused many of the remaining larvae to crawl off, fall into pans, and be recycled through the pupation room. Pupae were loaded into screen trays, stacked on racks, and moved through the holding room for 5½ days by monorail. After this holding period, they were either recycled to the adult colony or

loaded into stainless steel canisters and irradiated (before leaving the fly security area). After irradiation they were conveyed directly to the packaging area. Mechanical shakers have also been used to separate corn earworm pupae from trash (Sparks and Harrell 1976), and fruit fly pupae from vermiculite (Mitchell et al. 1965).

Sexing pupae

One of the most tedious materials-handling tasks required in rearing insects is the separation of pupae by sex. Several workable aids have been developed, but much data are needed to aid in the design of this equipment. A mechanical sexing aid (Wolf et al. 1972) was used to orient cabbage looper, *Trichoplusia ni* (Hübner), pupae for rapid identification through a microscope. A sizing machine developed by Schoenleber et al. (1970) used rollers to divide codling moth, *Laspeyresia pomonella* (Linnaeus), pupae into 10 groups by size as an aid in sexing them. Whitten (1969) reported mechanically sexing pupae of the Australian sheep blow fly, *Lucilia cuprina* Wiedemann, by differentiating between the light transmission of the male (both sexes are normally brown) and female (genetically induced black) pupae.

Sanitation in handling pupae

Sanitation procedures in pupal handling have included screwworm pupal trays being sterilized in a steam cabinet while still on the monorail (Goodenough and Brown 1976) and soaking of tobacco hornworm pupation units (Baumhover et al. 1977). Since protection provided by soaking in a sodium hypochlorite solution was short lived, the tobacco hornworm units were held for 2-3 seconds in a 1.5 g/liter solution of streptomycin sulfate to provide a residue of antibiotic.

Materials Handling for the Adult Colony

A special facility may be provided for maintaining an adult colony and obtaining eggs. It may include holding cages, feeding devices, oviposition stimuli, and equipment for disposal of spent insects and materials. Holding cages range in size from 11.4-liter cylindrical containers made of fiber and lined with paper (ice-cream cartons, see Reeves 1975 or Bell et al. 1981) to 1.2- by 1.8- by 2.4-m cages on rollers (Goodenough and Brown 1976). Some are equipped with special oviposition devices (see, for example, Baumhover et al. 1966, 1977; Barnes 1976; and Goodenough and Brown 1976).

The cleaning of holding cages may require them to be placed in a chillroom for immobilization of insects (Goodenough and Brown 1976); cleaning may also require special facilities for cleanup (Harrell and Griffin

1981) and equipment for disposing of spent adults and diet (Morrison and Hoffman 1976). Environmental control may include special equipment to remove dust and scales (Sparks and Harrell 1976); microbes (Harrell and Griffin 1981); or hair, scales, and moths (Bell et al. 1981).

Materials Handling in Packaging

Materials-handling procedures for packaging depend on whether individual or bulk handling is desired, stage of the insect, distance to be moved, and time needed for moving. An appropriate container can be designed when these and the range in ambient weather conditions are known. Richards (1961) expressed density of packing as numbers of nearest neighbors surrounding an atom in a crystal. The hexagonal close-pack configuration is the densest possible arrangement and has 12 nearest equidistant neighbors. Thus, Petterson and DeBolt (1976) reduced space needed to rear and transport cabbage looper larvae by using Hexcel trays instead of cups. McInnes et al. (1976) reported that tachinid puparia were held in individual gelatin capsules that, in turn, were placed in holes in polystyrene blocks; much hand labor was needed to place the puparia in the blocks; but moving, storage, shipment, and identification of emerged and hyperparasitized puparia was easy.

Ridgway et al. (1977) observed that bulk handling is desirable because it saves packages, labor, and space. Bulk handling was first applied to the screwworm and later, by Higgins (1970), to pink bollworm, *Pectiniphora gossypiella* (Saunders). Now it is also being used for other species. Screwworm pupae were shipped in bulk to packaging centers where machines formed the box and automatically dispensed measured amounts of pupae into it (Goodenough and Brown 1976). But adding a separator and food, closing the boxes and placing them on trays, and placing the trays on racks had to be done by hand. Wolf and Stimmann (1971) developed a machine for transferring live insects in bulk by a cyclone principle.

Baumhover et al. (1977) reported that vermiculite and several other packing materials were tested for shipping tobacco hornworm pupae and that "sandwiching tobacco hornworm pupae in a 12-layer absorbent paper cushion gave the best results (only 2.3% loss)." Tanaka et al. (1972a) reported that using evacuated polyethylene bags reduced the Mediterranean fruit fly's metabolic rate so that pupae remained at ambient temperature. In fact, emergence was delayed about 2 days, shipping costs were reduced from \$10 to \$0.50 per million, and only about half the original space was needed. The previous method was to ship pupae in shallow, screened containers placed in cardboard frames.

Improved packaging techniques have greatly reduced labor required to distribute a *Trichogramma* sp. egg parasite. Previously distributed by having parasitized Angoumois grain moth eggs put in small packages, *Trichogramma pretiosum* Riley has more recently been released in bulk. Reeves (1975) reported that a triangular paper package developed for packaging coffee cream was incorporated into a machine to mechanically release *T. pretiosum* from aircraft. Next, Angoumois grain moth eggs infested with *T. pretiosum* pupae were attached to bran flakes and dispensed with a ground broadcast unit (Jones et al. 1977). Later, Jones et al. (1979) and Bouse et al. (1980) reported bulk aerial release of infested eggs attached to bran flakes. Recently, cooled, gearmotor-driven units have been developed that allow bulk handling and distribution of the eggs from aircraft without any carrier material (Bouse et al. 1981).

Materials Handling in Distribution of Insects

Insects are usually moved from rearing facilities to distribution points by aircraft, especially if long distances are involved. Distribution may be by ground or aerial methods, but aerial release is preferred for large areas and if a high degree of release uniformity is required. Screwworm flies in boxes were released as soon as possible when 80% had emerged (Goodenough and Brown 1976). If release was delayed because of inclement weather, mechanical problems, etc., boxes were held at 13° C after the 80% level was reached. The boxes were metered from aircraft by a variable-speed conveyor. During long periods of inclement weather, flies could be released from open trucks.

A system for bulk release of screwworm flies that would use a whirling mass of air has been designed (vortex principle, C. Husman, personal communication), but not built. The U.S. Animal and Plant Health Inspection Service, Mission, Tex. (H. C. Hofmann and others, unpublished data) has developed and field-tested the release of chilled screwworm flies. The chilled-fly technique could be used in the regular program relatively quickly. Some research has also been done on the aerial release of screwworm pupae (A. B. Broce, P. Nichols, and associates, unpublished data), but additional technology will be needed if pupal releases are to be made regularly.

Aerial transport of screwworm pupae was reported by Baumhover et al. (1955) and by Coppedge et al. (1978). The melon fly, *Dacus cucurbitae* Coquillett, was transported to other programs by aircraft (Tanaka et al. 1972b). Other insects successfully transported by aircraft include tobacco hornworm pupae (Baumhover et

al. 1977); corn earworm pupae (Sparks and Harrell 1976); olive fruit fly, *Dacus oleae* (Gmelin), pupae (Remund et al. 1977); *Trichogramma* spp. in parasitized eggs (Ridgway et al. 1977); and eggs in bulk (Bouse 1981). Ridgway et al. (1977) also reported a free drop of *Lixophaga diatraea* (Townsend) adults. These flies, recovered after being chilled and then dropped from aircraft, lived as long as untreated flies.

Effects of Physical Properties of Materials on Handling

Physical properties affect the ease and method of handling and storage and the amount of damage that will occur during handling and storage. Generally, the greater a material's mechanical strength, the more it resists damage during handling. Mohsenin (1970) discussed various measurements and tests that can be used to describe the structure, physical characteristics (shape, size, volume, surface area, density, color, and appearance), and mechanical and rheological properties of plant and animal materials; the influence of these properties on texture of foods; and their relationship to aerodynamic and hydrodynamic characteristics and frictional properties of plant and animal materials.

Mechanical properties are those describing the behavior of the material under applied forces, such as stress and strain of a material under static and dynamic loading and those describing flow characteristics of a material in air or water. "Rheological" is a term used to describe mechanical properties when applied forces result in deformation and flow in the material. It is appropriate to describe diet materials and sometimes insect materials by either mechanical or rheological properties on the basis of the type of deformation that results from the applied loading. The factors involved in rheological behavior are force, deformation, and time. Properties used to express changes in materials that may result from these factors are time-dependent stress and strain behavior; creep; stress relaxation; viscosity; acoustics; electromagnetic (including dielectric and magnetic), photometric, solar, and ionizing energy; and effects of vibration. As an aid to developing mechanical devices for processing insects rapidly without injury, Stimmann et al. (1972), after studying the effects of simulated free fall on cabbage looper pupae, reported the need for additional studies to determine how rheological properties affect pupae, how abrasion affects pupae, and how the impacts of pupae affect surfaces softer than those used in the free-fall simulation tests.

Data on physical properties of materials are useful to designers of nearly all materials-handling equipment. The Standard Handbook for Mechanical Engineers (Baumeister et al. 1978) includes a guide for selecting

the type of materials-handling system and a table of what types of conveyors and elevators are best, depending on the physical conditions of the material. Data useful to formulating insect diets come from the food industry. Similar data are needed about physical properties of the insects; such data would be especially useful in designing equipment used when vibration or shocks are encountered (as during separation from pupation medium; during transportation to storage; during packaging and shipping; and in development of bulk-handling systems). Present data and the techniques for obtaining them are incomplete; so pioneering research is needed before an adequate data base will be available. Also important is that the data be reported in a standard format so that the values will be readily usable by others. (This was suggested by Mohsenin 1970; see also the Agricultural Engineers Yearbook, American Society of Agricultural Engineers 1982, for tables of "Preferred Units for Expressing Physical Quantities" and "Radiation Quantities and Units.")

Materials-Handling Needs

Great improvements could be made in materials-handling efficiency through alteration of the biological system. If, through genetic selection for instance, cannibalistic behavior in *Heliothis* spp. or tobacco hornworm larvae could be reduced or eliminated, rearing efficiency could increase by an order of magnitude or more. These insects could be used to produce beneficial insects and virus; and they might still be suitable for certain sterile-insect programs. Other basic simplifications would be enabled by changes in the actual diet materials, such as complete removal of agar from the gypsy moth diet to cut costs (a major problem cited by Bell et al. 1981).

Technology to automatically sex insects would greatly reduce labor required and permit expanded work in many programs. Rössler (1975) reported that sexing was required for strain research such as single-pair matings, rearing of hybrids, and field release of only one sex of Mediterranean fruit fly. Similarly, Baumhover et al. (1977) reported the need to sex pupae in rearing the tobacco hornworm. White and Mantey (1977) reported that sexing was required for codling moths for release-recapture experiments of irradiated and nonirradiated laboratory and native moths. The same needs were prevalent in the screwworm program. The immense task of manually sexing large numbers of irradiated flies needed for release and recapture of different sexes of flies in different areas has caused several studies to be abandoned by the U.S. Agricultural Research Service. The U.S. Animal and Plant Health Inspection Service in Mission, Tex., attempted to use the machine described by Schoenleber et al. (1970) to sex screwworm pupae by size, but this technique was unsatisfactory because groups of larvae

reared on different vats varied too much from one another. A similar problem was reported by Wolf et al. (1972) for cabbage loopers and by Carlton and Hardee (1974) for boll weevils and, in both cases, led to development of mechanical sexing aids for pupae. Bell et al. (1981) reported that a virus removes gypsy moth females biologically from some of their wild strains; but the virus is too detrimental to the mass-production system. Because female gypsy moths are about three times larger than males, separation on the basis of size is practical for that species. Godbee and Franklin (1978) reported that sexing of the black turpentine beetle, *Dendroctonus terebrans* (Olivier), by sound is possible because 87% of the males but no females make high-pitched chirping sounds. They reported that the regular method of sexing these beetles is by shape of the posterior seventh tergite but that extreme care must be exercised in sexing young adults in this manner because of possible injury.

Many aspects of handling in rearing should be evaluated in terms of quality control. Because this subject is treated in detail by others (see "Putting the Control in Quality Control in Insect Rearing," by D. L. Chambers and T. R. Ashley, and "The Closed-Loop System of Quality Control in Insect Rearing," by J. C. Webb.), I do not include it here.

A variety of other materials-handling needs have been cited by various authors. Bell et al. (1981) reported that automating the implanting of eggs on diet and the harvesting of pupae in the gypsy moth rearing program could reduce costs from \$12/1,000 insects to about \$7/1,000. Harrell et al. (1977) called for the establishment of optimal larval holding environments. They added that optimal shape, size, and wall thickness needed to be determined for larval rearing trays and that a mechanical system was needed for handling the processed trays through the larval stage and for harvesting and using the weevils.

Key needs in materials handling for the screwworm rearing program and other, similar programs are automatic feeding of larvae and removal of spent media from larval-rearing vats, mechanized handling of dry materials in the diet-preparation room, elimination of chilling of oviposition cages during cleanup, development of technology for a bulk-release system, application of linear programming to optimize planning or use of other techniques of operations research (see Gass 1958 and Hillier and Lieberman 1974; and see "Systems Analysis and Automated Data Processing for Quality Control . . .," by D. H. Akey, for an example of computerized management of data from an insect-rearing program), an improved quality-control program including feedback of field data to rearing (see "The Closed-Loop System of Quality Control in Insect Rearing," by J. C. Webb), computerized handling of field-evaluation

data, development of techniques allowing release of only one sex of insect in any particular area (by a biological or mechanical sexing method), further development of automated packaging procedures, and close attention to worker safety (see Snook 1978).

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The Closed-Loop System of Quality Control in Insect Rearing

By J. C. Webb¹

In recent years, insect rearing has changed from being mainly the maintenance of small laboratory cultures to operations producing millions of insects each week. With increases in both the number of insects reared and in the total cost of rearing them, it has become evident that good quality-control practices are needed. The objective of quality control is to insure that a product conforms to predetermined specifications or standards. To achieve this end, the desired characteristics must be known. Development of this information is the joint responsibility of engineers, entomologists, and administrators.

A systematic approach is essential in maintaining any good quality-control program (King 1975). Such an approach would include development of systems and techniques for conducting and interpreting the quality-control process. Many systems and techniques are available; they range from the simple (making an observation and pushing a button) to the complex (computer-controlled systems). But, regardless of its complexity, some basic elements are common to any system. Control over quality depends on the availability of pertinent information on which to base decisions. A quality-control system that regularly provides this information is a feedback or closed-loop system.

MacFarlane (1964) describes the feedback system as comprising four basic elements: the input, output, feedback loop, and comparator (fig. 1). The reference input is the standard that has been set for the product. A sample of the output is measured and the results fed back for comparison to the standard. Then, if necessary, adjustments are made in the error variable. In insect rearing (fig. 2), the main system would consist of all phases of the rearing process such as egg collection, larvae rearing, and pupal development. The output, adult insects, would be sampled and tests conducted on selected factors such as flight propensity, vision, sound, and mating ability. These results would be compared to the reference input, or standard; if they were within the tolerances, no change would be required in the rearing system. But, if one or all factors fell outside the specified limits, then some adjustment would be required.

The concept of a feedback system is not new; in fact, elements of it have been around for hundreds of years. It is used in most quality-control programs in industry and is adaptable for insect rearing, provided the appropriate input information is known. This kind of feedback system can be described by the general equation that

$$I(d^2\delta t^2) + B(d\delta/dt) + k\delta = (\delta i).$$

This general equation governs the behavior of any closed-loop system; the left side describes the output and the right side the input. The uniqueness of the equation to a specific system is in the coefficients (I , B , and k). These are factors that are fed into the system and also the output that is measured and compared to the standards. So values for the coefficients must be as accurate as possible. (See MacFarlane 1964 for a full discussion of this kind of equation.)

In insect rearing, the main closed-loop system is composed of many subsystems (fig. 3). Some subsystems depend on other subsystems; some are independent. The combined subsystems maintain each of the variables that directly affect quality of the adult insects (for example, pupal weight, temperature of larval medium, light level in egg-caging cages). The same general equations and feedback apply to the main system and its incorporated subsystems. For a system to be effective in feedback and control, we must know what factors are important and be able to measure them, set their tolerances, and collect data on them.

Selection of factors to be controlled is the first step in developing the quality-control system. This selection is done routinely in any insect-rearing program, whether systematically or not. The factors selected must have a direct relationship to the quality of the final product. For example, there are probably several points along the production line where the temperatures should be recorded and controlled. It may be important also to control light quality and quantity in the egg-collection cages. There may be other subsystems where pupal or larval size are important. We know that insects reared for a sterile-male-release program must be able to seek out and mate with their native counterparts. So they must be able to propel themselves from one location to another and also to perform the normal courtship ritual. Some of the measurable factors that may be important for sterile-release insects, then, are ability to fly, walk, see, call, and produce sounds. Once a factor has been identified and selected to

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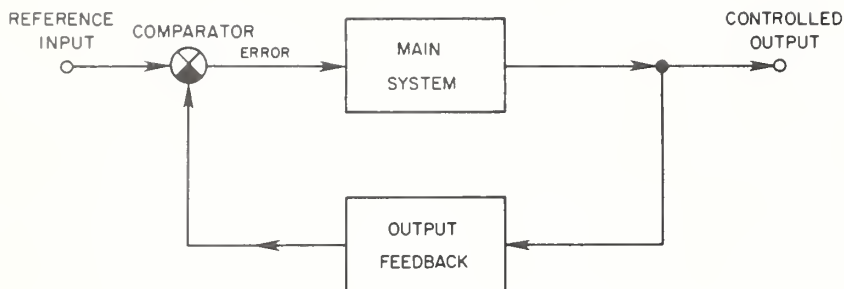


Figure 1.—The ideal feedback system.

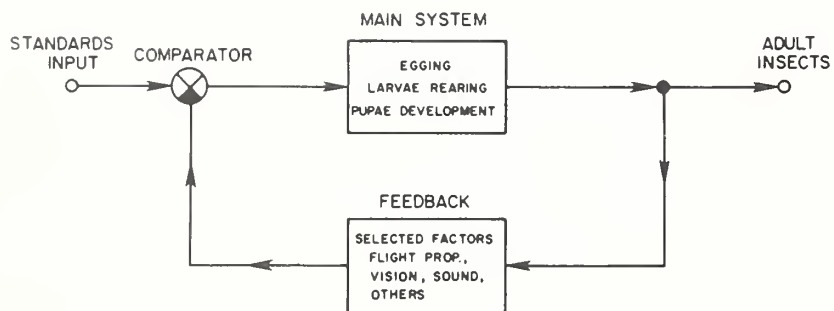


Figure 2.—Feedback system for insect rearing.

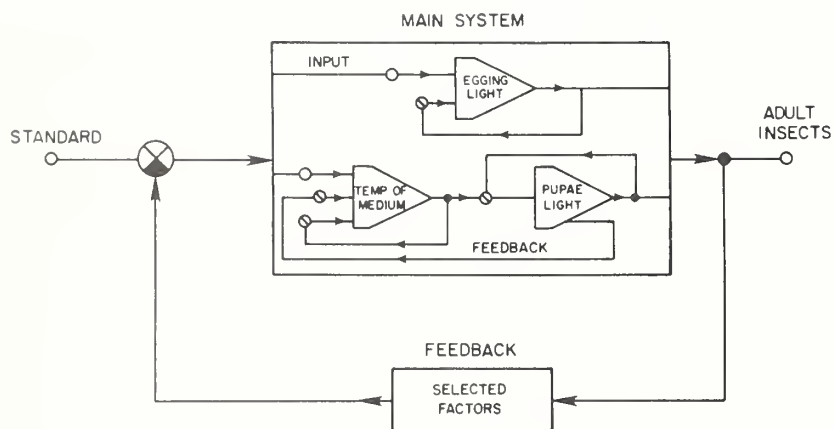


Figure 3.—A main insect-rearing system composed of several subsystems.

go into the system, its quantity and tolerances must be set. Each factor that is known to affect the quality of the final product should be included in the system.

Progress has been made in recent years in the basic studies of insect behavior and behavioral modifiers. From these studies have emerged some simple tests that can be conducted to measure aspects of insect behavior. Several of these tests are now included in the quality assessment of some mass-rearing and laboratory-rearing programs. Tests are being done of mating propensity, flight ability, flight rate, and pupal weight. Research is being conducted in many other areas. For example, sound is produced by many insects, and it has several measurable variables such as wing beat frequency, pulse information, and waveform distribution. Visual acuity is another factor that holds real promise as an indicator of insect quality. Other areas being researched are pheromone response, pupal size and weight, factors affecting reproduction, and factors affecting motility. There are two requirements

associated with factor selection; one, that the selected factors must be quantifiable, and two, that these factors must relate to the quality of the insect. Once these requirements are met, the selected factors can be included in the model.

This has been a discussion of systems theory. In an actual operation, many systems, simple or complex, have to be modified to fit the circumstances.

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Systems Analysis and Modeling in Mass Rearing and Control of Insects

By D. G. Haile and D. E. Weidhaas¹

Introduction

Systems analysis is applicable to practically any field of endeavor and certainly to mass rearing and control of insects. Systems analysis and modeling can be particularly useful in implementation of IPM (integrated pest management) procedures because the biological systems and control techniques are so complex. This paper is limited to application of the systems approach to modeling, or simulation, of insect life histories and population dynamics with references and examples from our work with mosquitoes. The basic techniques that we emphasize can be used as the initial stage in the modeling process for insect populations in general. These techniques can be expanded into very complex, computerized models of population dynamics. We do not try to present a comprehensive review of the many plant-pest models and computerized systems for implementation of large-scale IPM techniques.

Modeling can be used for learning and information assimilation, for focusing attention on the important variables and components of a complex system and promoting a better understanding of their interrelationships, for evaluation of knowledge limitations and research needs, for simulation experiments, and for prediction of system behavior under various conditions. These are not necessarily independent results and certainly do not include all possible uses of models. Various authors, such as Naylor et al. (1966), have discussed these and other potentially useful aspects of simulation studies in detail. The rationale for development of simulation models is generally obvious to most researchers; but in many cases, the full value or potential of these studies is not fully appreciated until one is directly involved in development of a fairly complex model that confirms existing data or theories. In general, the use of models is directly related to application of the scientific method. For example, when a system is so complex that simple observations cannot be made, models can be used to substitute for direct observations and aid in development of hypotheses. Likewise, model results can be compared to actual data in testing hypotheses. The concept of testing hy-

potheses is important when models for insect populations are considered, since data on the effects of several important variables will generally be limited or nonexistent. The modeling process can be used to combine existing data with estimates or questionable data on key variables. Using modeling in this way will encourage research on the most important variables where data are questionable, until models with sufficient complexity and accuracy for practical applications evolve.

Simplified Modeling Techniques

Perhaps the simplest modeling technique applicable to insect population dynamics and control uses a growth potential, or reproduction rate, per generation, R_o , and a factor representing the effect of a control technique, S , to relate the population density in one generation P , generally thought of as the parent generation, to the next generation, F . This relationship is

$$F_1 = P(R_o)(1 - S). \quad (1)$$

If no control is visualized, $S=0$ and

$$F_1 = P(R_o). \quad (2)$$

In this case, the actual change in density (F_1/P) is equal to R_o . When a control technique is applied, a distinction must be made between R_o and the actual growth rate or change in density ($R_a = F_1/P$). So

$$R_a = F_1/P = R_o(1 - S). \quad (3)$$

In a population with a growth potential of $5\times$ and a control action that is 90% effective for one generation ($S=0.9$), the actual growth would be 0.5 — $R_a = 5(1 - 0.9) = 0.5$ —and $F_1 = 0.5P$. The model can be used repeatedly for successive generations to establish the trend of a population over time for a given control measure. Knipling (1964) used this type of model effectively to illustrate the difference between the sterility and insecticidal approach to insect control.

Effective use of this type of model depends on a knowledge of the key variables, R_o and S . The accuracy required for the variable estimates depends on the purpose of the modeling effort and the uses to be made of the result. Intuitive estimates based on experience or limited data can be very useful in models to demonstrate the theoretical potential of a particular control procedure.

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But, for more critical applications or prediction of actual population changes, more precise estimates will be required, and the results will only be as good as the data used to develop the estimates. Estimates of R_0 for natural populations may be obtained when data on population density are available over a period of time and the generation time is known. In this procedure, the density ratios at generation intervals provide values of R_0 . Currently, many survey methods are used for sampling the various stages of insects, and a large amount of data is available. Unfortunately, much of this quantity of data is inadequate for use in analytical models. To be useful, the survey method must be applied consistently over a sufficient period of time, and the sampling efficiency of the method must be constant over time and for different locations. Also, the population being surveyed must be uncontrolled, or the effect of any control actions must be known. We should not be discouraged by the difficulty of obtaining meaningful data on these variables and abandon the modeling approach. Instead, we should continue to build a basic set of data that will allow the modeling process to become more and more practical.

The equations above represent a useful but elementary approach to modeling insect population dynamics and control. More complex models will be needed for the many systems and control measures required for IPM. The above equations, then, should be considered as a first step in a modeling process that can be made as complex as the system under consideration requires. An extension of the above modeling approach can be made by including additional variables related to the life cycle or life history of the insect under study. In a classic life-history analysis (Birch 1948), the reproduction rate (R_0) is related to survival and progeny production so that

$$R_0 = \sum (l_x m_x), \quad (4)$$

where l_x = probability of survival to age x from birth or oviposition
and m_x = female progeny produced per live female at age x .

This relationship can be used to calculate values of R_0 from normal life-table data. Another useful equation for mosquito populations and several other insects can be derived from this relationship by applying certain generalizations common to many insect life histories (Weidhaas and Haile 1978). First, since oviposition occurs only in the adult stage, immature survival can be included as a separate variable. Then, assuming that the rates of adult survival and oviposition are constant with age and that oviposition occurs at discrete intervals after a preoviposition period,

$$R_0 = S_i(m)(S_a^d/1 - S_a^c), \quad (5)$$

where S_i = probability of survival from egg to emerging adult,
 S_a = average daily survival rate of adult females,
 m = average number of female eggs per live female per oviposition,
 d = preoviposition time in days,
and c = laying cycle in days.

In this equation, S_i can be subdivided into age classes if desired. For example, if the development time (in days) for eggs, E ; larvae, L ; and pupae, P , are known and a daily survival rate for each stage (s_e, s_l , and s_p) is known, then $s_i = (s_e^E)(s_l^L)(s_p^P)$. Also, if a daily survival rate, s_i , is given for the complete immature development period, I , then $S_i = s_i^I$. As a numerical example for equation 5, consider a mosquito population where $S_i = 0.853$, $S_a = 0.85$, $m = 60$, $d = 6$, and $c = 3$. Then $R_0(0.853)(60)(0.85^6/1 - 0.85^3) = 5$. Again, a model such as this is no better than the estimated variables that are used. And, in most cases, field data on these variables may not be available. But the method is available for theoretical calculations and will be more applicable for practical field problems as the data base on field populations is accumulated. Currently, good estimates can be made for values in this equation for many insect species. Note that the values for R_0 in equation 5 can be used in equations 1 and 3 to calculate density trends for control measures that limit the reproduction rate.

Since equation 5 includes survival rates, we can make theoretical calculations from it about other control techniques that might affect the survival rate of specific stages. The effect of a control technique must be expressed as a constant daily mortality, M , in specific daily age classes in the insect life cycle. Determining M is possible for many control techniques, such as continuous treatment with biological agents, insecticides, or traps that kill a constant proportion of given stages. For a given M , a survival rate due to control, S_c , can be calculated ($S_c = 1 - M$) and used in equation 5 to reflect the actual growth rate, R_a , after the control technique is applied. For control techniques that affect the adult population, where S_a is already a daily survival rate, S_a would be replaced by $S_a \cdot S_c$ and

$$R_a = (S_i)(m)(S_a \cdot S_c)^d / 1 - (S_a \cdot S_c)^c. \quad (6)$$

In the case of control techniques that cause deaths in the immature stages, S_i would be multiplied by S_c^N where N represents the number of daily age classes affected by the control. So

$$R_a = R_0 S_c^N. \quad (7)$$

For example, consider a mosquito population where the egg, larval, and pupal development times are 2, 6, and 2

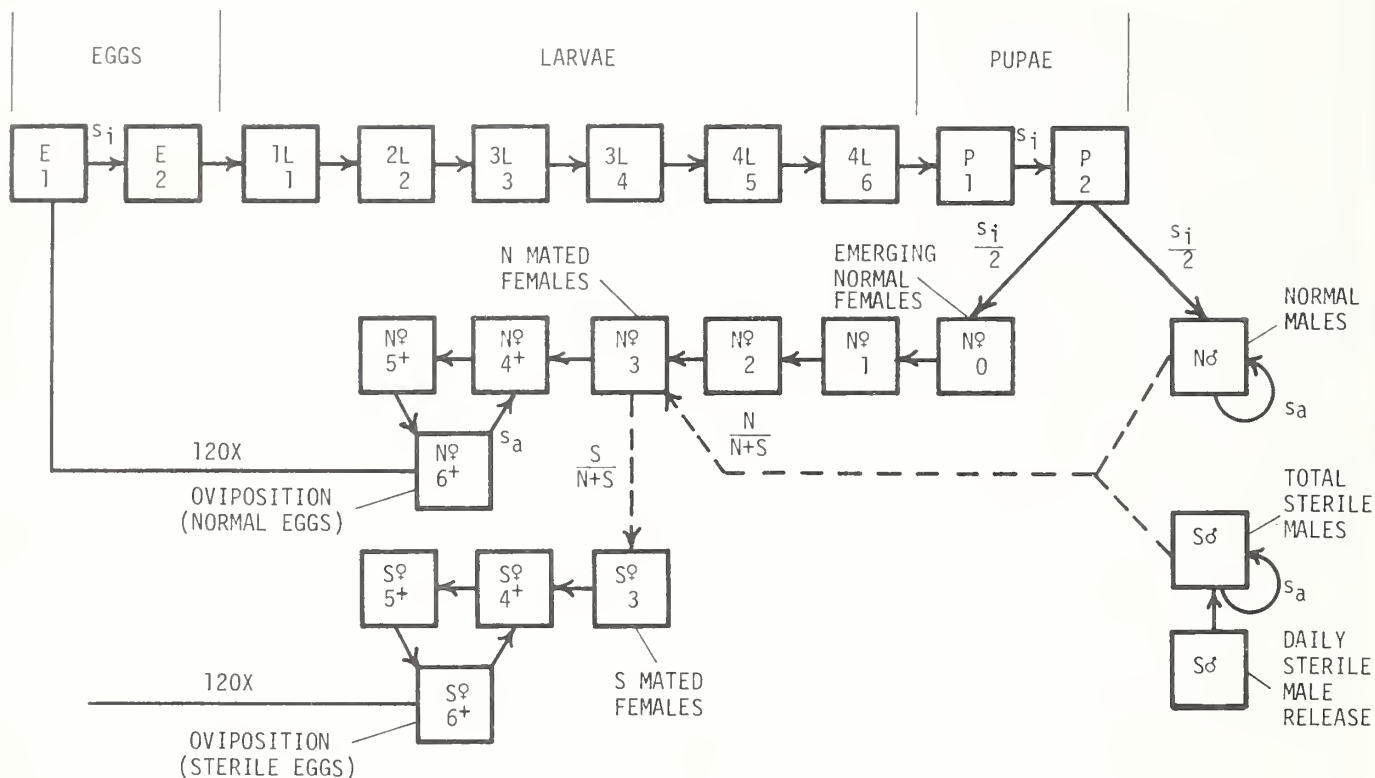


Figure 1.—Diagram of a life-history model of a mosquito with 1-day age classes, including the effect of sterile-male release. (Stages include: E =eggs; $1L$ =first-instar larvae; $2L$ =second-instar larvae; $3L$ =third-instar larvae; $4L$ =fourth-instar larvae; P =pupae; N =normal adults; S =sterile adults.)

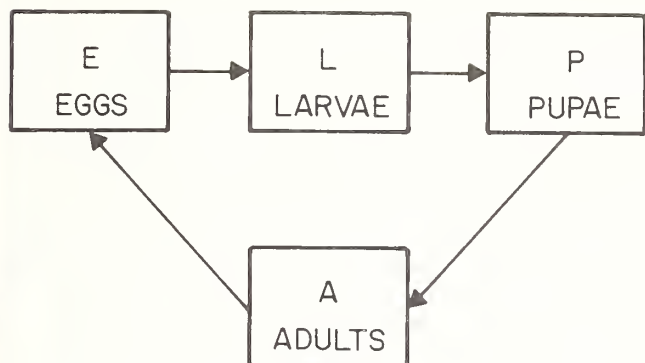
days. If the control affects all larvae equally, then $N=6$ and $R_a=R_0S_c^6$. Likewise, if the control affects all eggs or pupae, $R_a=R_0S_c^2$. If S_c affects only one age class (1 day), such as the last day of larvae, then $R_a=R_0S_c$.

These modeling techniques allow us to make theoretical calculations interrelating dynamics and control from one generation to the next. But the mathematics for these equations require that daily average mortality or survivals be used. Unfortunately, many control agents are not applied every day or for some other reason prevent the use of average daily survival or mortality. So more complex modeling techniques are required to give added realism to theoretical calculations and to simulate the actual effects of complex IPM control strategies more accurately.

Computer Simulation Models

Computer technology can be used to extend the complexity and versatility of the life-history approach to modeling of insect populations. Munro (1973) pointed out that development of dynamic life-table models represents the

most direct application of computers to insect populations. Basically, this technique involves use of a computer to store an insect life table, which represents the number of individuals present in each age class for a given unit of time. A computer program can then be written to calculate changes in numbers and age class, for each successive time unit based on survival rates and information on progeny production from a given initial population. Haile and Weidhaas (1977) used this approach for a computer model of a mosquito (*Anopheles albimanus* Wiedemann) population with daily age classes. This model (fig. 1) also included provisions for simulating control by release of sterile males. Other control techniques can easily be programed for this type of model by establishing how the technique affects the number of individuals in given stages, the survival rates, or fecundity. The structure and complexity of this type of model can be modified to fit many insects and simulation situations. But its realism is limited mainly by the use of fixed developmental rates and discrete transfers between age classes (where all individuals of the same age change to different stages at the same time).



$$E_{t+1} = E_t - E_t M_e - E_t Q_e + A_t Q_a$$

$$L_{t+1} = L_t - L_t M_l - L_t Q_l + E_t Q_e$$

$$P_{t+1} = P_t - P_t M_p - P_t Q_p + L_t Q_l$$

$$A_{t+1} = A_t - A_t M_a + P_t Q_p$$

Figure 2.—Elementary insect life-cycle model that used a mortality factor, M , and a flow rate, Q , to establish the proportion of individuals transferring from one stage to the next during one increment of time, t .

Several approaches have been used to incorporate variable developmental rates into population models. Generally, the rate of development is a function of temperature and can be expressed in different ways, such as the degree-day approach. Models of the type represented in fig. 2 use development data to establish the flow rate of individuals, Q , from one stage to the next during a given time unit, t . This type of model is simple to use and easy to program in a computer. Also, the structure is amenable to various specialized simulation languages that allow very complex and versatile models to be implemented easily. This type of model is mainly limited by the fact that age of individual insects in the various stages is not differentiated; so a realistic representation of the development of one cohort of insects is difficult. Modifications, such as an increase in the number of stages considered, can reduce this effect; but increasing the number of stages results in models similar to the dynamic life table discussed above. A model using a dynamic life table can be modified to include variable

development and still realistically simulate the development of one cohort as well as overlapping generations. Fine et al. (1979) developed such a model by varying the length of time units with the development rate of the insect. And we are developing a dynamic life-history model that allows age within stage to vary according to accumulated development, which is temperature dependent. A similar approach is being used for stable fly, *Stomoxys calcitrans* (Linnaeus), populations by I. L. Berry (personal communication).

Basic life-cycle models of insect populations can be the framework for more comprehensive models that have many relationships between environmental variables (such as temperature, humidity, rainfall, and habitat) and insect variables (such as development, mortality, predation, parasitism, fecundity, and density). Many of these relationships are complex, and the insect variables are generally interrelated. Also, models of crop pests generally include some form of a model of plant development (see, for example, comprehensive plant-pest models, for cotton insects, reported by Hartstack et al. 1976 and Fye 1979). The various approaches for such models depend largely on the model objectives and the available or expected data base.

Conclusions

Computer simulation is a valuable research and analytical tool that can and should be used in the development of insect-control technologies, including those that depend on mass-reared insects. Models provide valuable insight into complex systems and promote development and testing of theories. Because of the complexity of the systems involved in insect control and the difficulty of obtaining adequate data, an interdisciplinary team approach is essential to effective development and use of models. Effective model usage also depends on our ability to identify appropriate problems, establish objectives, and allocate sufficient resources. With enough field validation, population-dynamics models can be used in practical IPM programs for predictions of population levels and timing of appropriate control actions.

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Section 4

Control of Pathogens and Microbial Contaminants in Insect Rearing

In general, disease has been one of the greatest impediments to successful mass rearing of insects, and much of the history of insect pathology is centered on diseases of two economically important insects: the honey bee, *Apis mellifera* Linnaeus, and the silkworm, *Bombyx mori* (Linnaeus). The intent of this section is to emphasize the impact of micro-organisms on insect cultures and the measures available to minimize or eliminate the pathogens or contaminants. Special attention is devoted to the recognition of diseases (and micro-organisms) in insect rearing since the success of a given control measure may depend on the micro-organism involved.

In a rearing facility or insectary, the sources of contaminants are numerous and include the diet, the insect, the rearing facility, and the rearing personnel. The sources of microbial contaminants are described here in detail as are measures that may be used to minimize or eliminate the micro-organisms. In many instances, descriptions of control measures include discussion not only of how they affect contaminants and pathogens but also of how they affect the insect.

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Recognition and Diagnosis of Diseases in Insectaries and the Effects of Disease Agents on Insect Biology

by Ronald H. Goodwin¹

Introduction

Diseases, or any of several other possible departures from the normal healthy state, are almost certainly more common among insectary-reared insects than among insects that develop in the field. This is to be expected, since an insectary environment can never be an exact duplication of field conditions and usually involves a degree of insect crowding that never occurs in the field. We must assume that any artificial rearing system produces a definable stress on the confined population; and we know that environmental stress either causes disease directly or increases susceptibility to disease. Invertebrates such as insects have a rather rudimentary immune system, and they usually depend on population dispersal to remain free of disease. So insectary confinement insures that any communicable disease agents present will have the best chance possible to spread in the population. The stress of the rearing environment insures that individuals contacting such agents will be easily and rapidly invaded by any pathogens present in the field-collected starting stock or in the insectary itself.

Some of the insect diseases to be described and understood earliest were, not surprisingly, from the first two major insect-rearing systems, apiculture and sericulture. Sericulture was the source of the first scientific demonstrations of the disease process and of the importance of microbes in diseases; it was also the basis for the development of Koch's postulates (Steinhaus 1949). But most insect diseases are still unknown, and results of many present research programs involving reared insects have been compromised by these undiscovered diseases, especially the enzootic diseases, in the experimental stock. Where such diseases have gone undetected, published results contain wrong conclusions about basic biological descriptions, differential competitiveness, climatological resistance, parasitoid adaptability and effectiveness, and other factors such as insecticide resistance and sterile-male viability. Some recent rearing programs

have included disease prevention, monitoring, and elimination only after costly failures and much wasted effort.

Many of the major disease agents were originally found among reared insects rather than in the field. For example, the widely used and highly lethal HD-1 strain of *Bacillus thuringiensis* Berliner was discovered in an insectary for pink bollworms, *Pectinophora gossypiella* (Saunders). Because of stock crowding and the other inevitable stresses of suboptimal artificial diets and environmental conditions, insectaries have been a unique source of new pathogens. The more lethal agents are relatively easy to determine and eliminate from insectaries. But the enzootic or debilitating diseases are usually more of a problem since they often escape discovery until an insectary-based research program is well underway or, occasionally, even after it has been completed.

Pathogens such as the microsporidia, which apparently produce insect hormone analogs, have no doubt compromised many physiological and basic biological studies where their presence has gone undetected. Indeed, the microsporidia are probably the most common undetected pathogens; they are often transmitted transovarially and may persist indefinitely in a stock colony without ever quite destroying it. Meanwhile, they produce subtle and sometimes confusing effects in the host population.

Where imported foreign insects are being quarantined before release as weed-control agents or as parasitoids for insect control, particular care is necessary to free the initial stock from whatever pathogens are present (Bucher and Harris 1963). Diseased insects of foreign source may not be able to survive when exposed to a new climate and to field ecological systems that are inevitably alien to them and therefore initially stress producing. As a result, promising species may be prematurely abandoned because they were not disease free when introduced. (It would be counterproductive to introduce pathogens of natural enemies simultaneously with attempts to introduce and establish the natural enemy.) Conversely, some introductions may have failed because the stock was without its associated symbiotic micro-organisms (that is, were rendered aposymbiotic: Krieg 1971b, Boush and Coppel 1974). For example, Stoltz and Vinson (1979) discovered that certain baculoviruses present in the

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oviducts of all investigated braconid and ichneumonid parasitoid wasps may suppress the encapsulation reaction or otherwise modify the physiology of the usual wasp hosts; these viruses, therefore, must not be lost from introduced foreign stock insects through improper handling if we expect the insects to adapt successfully to their pest hosts in the new habitat. In general, though they are widespread in the Insecta, not much is known about symbiotic micro-organisms in insectary insects or their potential for insect control (available studies include Roth and Willis 1960, Brooks 1963, Buchner 1965, Batra 1979, and Houk and Griffiths 1980).

Also, where an investigator is concerned primarily with importation or colonization of insect predators or parasites, monitoring of the condition of the prey or host species may often be inadequate. Some host diseases do not seem to affect even internal larval parasitoids, which emerge as apparently normal adults; more often, the parasitoids are physiologically impaired to some degree. Again, such relationships have not been studied adequately.

Programs involving the release of insects that are sterile or contain a lethal gene depend on the health and competitive ability of the production stock. So the insectary should be able to produce a disease-free, physiologically superior sterile insect since it would have a critical and decisive advantage when the target adult field population is large (Hutt 1979).

While individual rearing may be too time consuming for regular practice, it should be used initially for at least two or three generations so that the introductory insectary quarantine will be effective. After the newly collected insects have been isolated and observed in a separate quarantine room and adequate procedures have been developed to prevent cross infections from occurring in the insectary (see "Micro-organisms as Contaminants and Pathogens in Insect Rearing," by Martin Shapiro), we may assume that the more obvious diseases have been separated from our insectary seed colony. While some diseases are highly lethal or otherwise readily obvious, most diseases are not so easily recognized, and many are quite cryptic. Insects that are apparently healthy may often carry these cryptic or enzootic disease agents, spreading them through contact or contaminated frass or scales throughout the colony where insects are reared in groups. Only through close monitoring of the colony, both macroscopically and microscopically (by sacrificing unusual or excess specimens), will clues emerge that indicate the presence of such diseases. Once the offending disease has been recognized in individually-reared seed-colony stock insects, it should be eliminated through careful destruction of the insects containing it, followed by the rigorous disinfection of contaminated cages, equip-

ment, and insectary environment (see "Micro-organisms as Contaminants and Pathogens in Insect Rearing," by Martin Shapiro). It is usually not possible to separate diseased insects from their pathogens. Such attempts are to be generally discouraged (but see Vavra and Maddox 1976 for microsporidia control).

Disease Recognition

Since the objective of the insect culturist is mainly the exclusion of disease (see Steinhaus 1953, Helms and Raun 1971, and "Micro-organisms as Contaminants and Pathogens in Insect Rearing," by Martin Shapiro), he is justifiably more interested in disease recognition (used here to mean identification of the causal agent) than in the more complex study of disease diagnosis. The insectary environment and the artificial diet provided are often the primary predisposing factors for any given disease. Therefore, here I will discuss in detail only the diseases most likely to be a problem in an insectary or in a similarly artificial insect-production system. Other disease factors will not be considered; but they must be taken into account in any final diagnosis decision, which must include all related factors, not just apparent primary pathogens. For such additional information and help in specific areas of disease diagnosis, refer to the following authors: Ainsworth (1971), a dictionary of fungi; Batra (1979), insect-fungus symbiosis; Boush and Coppel (1974) biology of symbiotes; Brooks (1963), micro-organisms of healthy insects; Buchner (1965), endosymbiotes; Burges (1981), eight chapters on the identification of insect pathogen groups include three on bacteria, one on viruses, three on fungi (with keys), and one on microsporidia (with a key); Cantwell (1974), honey bee diseases; Davidson (1981), symptomatology, pathogenesis, host resistance, and disease cycles of pathogens and toxins in insects and other invertebrates; Kramer (1976), dissemination of microsporidia; Lipa (1975), diagnostics and prevention of insect diseases, chapter 4; Longworth (1978), small isometric viruses of invertebrates; Maramorosch (1977), photomicrographs of viruses; Morse (1978), honey bee diseases including viruses, rickettsiae, bacteria, protozoa, and fungi; Müller-Kögler (1965), diagnosis of insect fungus pathogens, pp. 67-75; Nickle (1974), nematode infections and biology of nematode families, with many photographs; Poinar (1975), manual and host list of insect-nematode associations, with illustrated keys to families and genera, plus nematode study techniques; Poinar and Thomas (1978), identification techniques for fungi, bacteria, viruses, protozoa, and rickettsiae, with keys to groups and common genera, representative photomicrographs of all pathogen groups mentioned; Roth and Willis (1960), cockroach microbial relationships including symbiotes; Shimanuki and Cantwell (1978), diagnosis of honey bee diseases; Sprague (1977a), current classification of the microsporidia; Sprague (1977b), annotated list

of species of microsporidia; Sprague (1977c), zoological distribution of microsporidia; Steinhaus (1949), symptoms and pathologies, chapter 8, illustrations from many microbial groups; Steinhaus (1963), background for diagnostic work plus a complete step-by-step guide to diagnostic procedures; Steinhaus (1964), definitions in diagnosis with a discussion of the history and present unfulfilled requirements for adequate diagnosis of invertebrate diseases; Steinhaus and Martignoni (1970), glossary of invertebrate pathology; Thomas (1974), a general guide to the diagnostic equipment and procedures necessary for each major pathogen group; Torre-Bueno (1973), glossary of entomology; Vavra and Maddox (1976), the examination of insects infected with microsporidia and several techniques in investigating the microsporidia, with a short section outlining the control of microsporidial infections in insects; Weiser (1961), microsporidia in insects, contains many life-cycle drawings and photomicrographs showing the developmental stages and spores of many of the known microsporidians; Weiser (1969), a collection of photomicrographs of the many agents infectious to insects, with a few photographs of diseased whole larvae; Weiser and Briggs (1971), general keys to pathogen groups, to genera, and to some species based on gross and microscopic appearance of diseased specimens and the appearance of diagnostic pathogen stages, dimensions of micro-organism groups—table 1, (few photographs, keys to groups and genera partly outdated); Whitcomb and Williamson (1979), insect pathogenic mycoplasmas and spiroplasmas.

Steinhaus (1964) defined symptoms and signs of disease in insects to include specific and distinctive factors. Disease symptoms include abnormal movements, abnormal responses to stimuli, abnormal functional development, abnormal body rhythms, digestive disturbances, reproductive disturbances, variations in longevity, and odors. Disease signs include discoloration and alteration of color pattern, abnormalities of body size (and the size of parts), abnormalities in body shape, abnormalities in external structures, abnormalities in texture and sculpturing, abnormalities in consistency, traumata and wounds, prolapses and hernias, and observable presence of the pathogen (either macroscopically or microscopically). In many diseases, these indicators overlap, so care must be taken to overlook nothing that could lead to the correct identification or diagnosis of a given disease.

The first step in diagnosis is to make an external examination. This is followed by an internal gross dissection under a dissecting microscope; the insect is cut open with a midventral incision and laid out flat with pins so the internal anatomy can be seen and studied easily (this is termed an open dissection).

To make useful fresh wet-mount smears, use only a small amount of tissue. Suspend it in water on a glass slide and gently crush the sample with a coverslip placed on top. The aim is to break up the tissue into individual whole and broken cells. In some cases (for example, blood and fat body), the coverslip pressure alone is sufficient to adequately flatten and spread the cells of a small lump of tissue so associated microbes can be viewed clearly with phase objectives. Many pathogens, such as the larger coccidian and gregarine cysts and spores, need no more treatment than this. Accurate pathogen identifications can be made from photomicrographs of such preparations if the magnifications are recorded.

There are several simple-to-complex insect diagnostic histological and staining techniques (see, for example, Weiser 1961, Müller-Kögler 1965, Weiser and Briggs 1971, Cantwell 1974, Thomas 1974, Lipa 1975, Vavra and Maddox 1976, Poinar and Thomas 1978, and Shimanuki and Cantwell 1978). Of the many techniques available, the most generally useful are: (1) fresh wet-mount smears of blood, fat body, gut, and other tissues (in that order) observed under high-power and oil-immersion phase objectives and (2) Giemsa-stained smears of the same tissues. If there is an apparent fungus infection, Guegen's solution is recommended for help in identification (Thomas 1974, Poinar and Thomas 1978). Or lactophenol cotton blue may be used for fungi or for the entomopoxviruses (Poinar and Thomas 1978; see the preparation technique for this stain given in the Entomopoxvirus "Microscopic Examination" section.

Either Giemsa powder (Sigma Chemical Co., St. Louis, Mo., stock No. G4507) or Giemsa stock solutions are widely available for use with the Giemsa stain procedure. If Giemsa powder is used, dissolve 1.25 g of powder in 41.7 g of glycerol (warm glycerol to 60° C and shake thoroughly to dissolve the powder). Add 125 g of absolute methanol, shake to mix, and allow to stand overnight at room temperature. Filter with a No. 2 Whatman filter in a vacuum-operated Buchner funnel. This solution will store indefinitely if water is not added. For a repeatable stain reaction, dilute 1 : 20 (or 1 : 25 when the technique concentration is listed as 1 drop/ml of water; this equals 4 ml stain stock plus 100 ml of water or buffer) with buffer ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 1.707 g; KH_2PO_4 , 1.88 g; distilled water, 500 ml), not with water. The stain is unstable when combined with the buffer (or water) and should be discarded within 24 hours. Air-dried thin smears on glass slides are first fixed in methanol for 3–5 minutes and then stained in 1 : 20 Giemsa buffer for about 1 hour (try more or less time depending on results). Wash smear gently with buffer or distilled water and blot or air-dry. Giemsa stain modifications and the expected results with each pathogen group are indicated in the sections on microscopic examination.

The key below (key 1) to the insect diseases is designed for use with living insects in an insectary or under conditions where continuous observation of living specimens is possible. Starvation, asphyxiation, overheating, drowning, wounding (including gut or integument breakage), and poisoning or other chemical assaults must be separately considered before use of this key (Steinhaus 1953). Dead larvae will usually contain several bacterial types; once bacterial rotting has occurred, the correct identification of the primary pathogens that may be present is usually more difficult. Although the described diseases are usually found in larvae, some of the same conditions may be found in adults of the holometabolous insects and in any growth stage of heterometabolous insects. Where open dissections are described, dissection of a normal larva should be performed at the same time for a direct comparison of the condition of the internal tissues.

Key 1.—Symptoms and signs of insect disease as seen with the naked eye or a dissecting microscope below $\times 80$

1. Stunted larvae, abnormally whitened or yellowed 2
 - Stunted larvae, no discoloration 3
 - Normal-sized insects showing nervous activity and trembling followed by lack of coordination and paralysis Picornaviridae
 - Normal-sized insects showing abnormal regurgitation and anal discharge (diarrhea) 4
 - Normal-sized insects showing discolorations (yellow, pink, red, purple, brown, green, blue) 5
 - Insect fails to molt or pupate normally 6
2. Light-colored stunted larvae (open dissection):
 - With midgut abnormally whitened Reoviridae or microsporidia
 - With fat body abnormal in appearance (distended, fragile, fragmented, or discolored) *Entomopoxvirus*, microsporidia, Neogregarinida, or Baculoviridae
3. Stunted larvae (no other signs or symptoms) *Chlamydia*²
 - Stunted larvae showing both regurgitation and anal discharge (sometimes with violent muscular contractions) *Enterella* (rickettsia)
 - Stunted larvae showing white fecal exudate microsporidia
4. Sawfly larvae showing white regurgitate (open dissection reveals abnormally whitened gut) Baculoviridae
 - Insects showing both regurgitation and copious anal discharge (death may occur in hours after symptom appearance) Picornaviridae
5. Pronounced or subtle color changes followed by solidification (mummification) at time of death (aquatic insects do not solidify) fungi
 - Subtle color changes followed by liquefaction of larval contents after death Baculoviridae (nuclear polyhedrosis)
 - Color changes followed by rotting of cadaver with sweet or putrid odors bacteria
 - Color changes persisting after death (decomposition may be delayed) *Rickettsiella* or Baculoviridae (granulosis)
 - Larvae with transparent cuticles may show progressive translucence of normally white internal organs, finally becoming a dirty mottle brown Coccidia (protozoa)
6. Insect becomes abnormally swollen-elongate (often lighter-colored than normal) *Entomopoxvirus*, *Rickettsiella*, Baculoviridae, or microsporidia
 - Insect dies while molting or pupating or emerges as a deformed adult (chalky appearance) Neogregarinida (protozoa)
 - Insect dies while molting or pupating, then darkens and rots (sweet or putrid odor) bacteria

²See "Rickettsiales and Chlamydiales"; see also "Note Added in Proof," p. 117.

Protozoa (and Microspora)

The one-celled animals, or protozoa, until recently contained among them the subgroup Microsporidia, which seems to be the most important and widespread group of pathogens found in insectaries. While Sprague (1977a) has placed these microbes in the separate phylum Microspora, this classification may or may not be accepted in the future. They will be considered as protozoans here for convenience since all of the earlier literature treats them as protozoans. Microsporidia and other protozoan pathogens are usually enzootic in field populations. Insects infected in the field or in the insectary may survive to reproduce and spread the infection throughout the environment. Often the signs and symptoms are obscure, especially in insects not having a transparent cuticle. While originally thought to be rather host specific, protozoan species in the microsporidia, Coccidia, and Neogregarinida are now known to be able to infect at least several related insect species and, in some cases, to cross familial or ordinal host divisions. Indeed, some microsporidians are now known from parasitic Hymenoptera (Brooks 1974), which implies that their elimination from introduced parasite species could be a practical means of improving parasite activity in new habitats.

Microsporidia (Microspora)

Disease cycle.—After microsporidian spores (often from fecal material of infected insects) are ingested, each forcibly extrudes a hollow polar filament that injects the first-stage sporoplasm into the gut cell; the sporoplasm grows intracellularly into the intermediate stage, a meront. Meronts divide to give further meront stages or sporonts and sporoblasts, which develop into mature spores. The meronts may be restricted to the gut or may pass into other tissues. Specific infections may then be characterized in part by the presence of meront stages and/or spores in certain tissues of the host. Many are confined to the gut and/or the malpighian tubules; others can be found specifically or more generally infecting the fat body, tracheal matrix, hypodermis and connective tissue, blood cells, silk glands, muscles, or gonads. When the infection is confined to one or two of these tissues, separate tissue dissections are the only way to confirm the microsporidian presence if a whole-body smear (possible with small larvae) is not feasible.

Lysed gut-cell contents release spores from the gut cells that survive in the fecal material or in regurgitate. Spores may also escape from the infected insect via egg-cementing fluids (in female lepidopterans), on the ovipositors of parasitoids, through cannibalism, or through the rupture of the infected cadaver and subsequent environmental contamination. Vertical passage of nonsporulated stages

and spores commonly occurs within the eggs of many invertebrates (Brooks 1974, Kramer 1976, Vavra 1976).

Symptoms and signs.—According to Vavra and Maddox (1976), although most infections outwardly demonstrate no characteristic signs or symptoms, transparent insects infected with microsporidia are often wholly or partly milky white within because of spores and growth stages packed into the infected tissues (for example, fat body, hypodermis, tracheal matrix, or blood cells; figs. 1A–1D). Where the insect is not transparent, a gross dissection may reveal a white distended gut or similarly discolored malpighian tubules or other tissues as compared to a similar dissection of a healthy individual (see Cantwell 1974, fig. 11). Infected larvae may show black spotting (fig. 1E), but such spots may also indicate a fungus or rickettsial infection. The infected insect may be stunted (fig. 1F) or, if molting is delayed, may become an abnormally large larva or a malformed pupa (figs. 1G and 1H). Sluggishness is common, as are uncoordinated movements, abnormal postures, and movements such as twitching. Infected individuals may reduce their food consumption or produce a white fecal exudate. Reproduction rates may or may not be measurably affected.

Microscopic examination.—The intracellular developmental stages of the microsporidia are readily stained with the Giemsa technique (appearing globose, with blue cytoplasm and red, compact-to-vesiculate nuclei; often with paired nuclei in meront stages), but mature spores will remain unstained. To stain the sporoplasm within the spore, the smear preparations must first be hydrolyzed for 10 minutes by treatment with 1N HCl at 60°–70° C, washed several times with distilled water, and then stained. The spore will then show a central or partly polar cytoplasmic blue element within but will not take the stain uniformly throughout. Oil-immersion observation with white light is required because of the small size of most spores. Consult the illustrated references (Weiser 1961, 1969 and others) for the appearance of the many types known. The most recent key was written by Hazard, Ellis, and Joslyn in Burges (1981, chapter 9).

Coccidia

Disease cycle.—Commonly, several nearly spherical coccidian spores (or sporocysts) are released from a globose-walled oocyst into the environment and are then ingested by susceptible hosts. The spores burst in the gut, releasing many sporozoites that penetrate and multiply in the gut epithelium or pass through and multiply in the fat body. The many asexually formed, wormlike meront stages eventually differentiate, pair sexually, and finally give rise to sporoblasts that develop into the final oocyst stage. Spore stages are released into the environment

through the ruptured cadaver or are spread by cannibalism in some species (Brooks 1974), but they are most commonly distributed with the feces in gut-specific species (Weiser 1963).

Symptoms and signs.—Heavily infected larvae may move more slowly than normal larvae, and their tissues are more frail (for example, in *Adelina tribolii* infections). In *Adelina sericesthis* infections, intermediate stage development induces an externally visible translucent clarity (in larval hosts with a transparent cuticle) in portions of the body where the fat-body tissue has been heavily infected (fig. 1I). Later infections in the same insects (near-moribund hosts) color the integument a mottle-brown (Brooks 1974), making them look as if they were filled with vermiculite (fig 2A); in late *A. sericesthis* infections, both the fat-body and the subcuticular connective tissue are infected and turn brownish as the mature oocysts are formed. One coccidian species that infects the malpighian tubules causes a clarification of that tissue. Reproductive rates may be affected in populations having sublethal infections. Insect-infecting coccidia are not host specific, but the host ranges of the known species have not been fully described.

Microscopic examination.—The intracellular developmental stages are easily stained with Giemsa, but the fresh spores are very stain resistant. Fortunately, the characteristic bag-of-balls oocyst structure is readily apparent in wet-mount smears of gut or fat-body tissue viewed with phase optics (see Weiser 1969, figs. 315–317).

Neogregarinida (Schizogregarinida)

Disease cycle.—The ovoid-to-spindle-shaped neogregarine spores are usually ingested in contaminated food. The spore wall ruptures in the gut, releasing many elongate, wormlike sporozoites that penetrate the gut epithelium where they either multiply or pass through to multiply in the fat body or malpighian tubules. One species is known to multiply in the hypodermis. First, a large multinucleate plasmodium is formed; from this, nuclei bud off and form elongate epicellular merozoites or globular intracellular merozoites. These further divide to fill the infected tissue with prespore sexual stages that unite to form the final spores. The spore stages are released from the host via the feces in gut or malpighian-tubule-specific species and via ruptured cadavers and cannibalism in fatbody- or hypodermal-cell-specific species.

Symptoms and signs.—Larvae infected with the neogregarine *Mattesia grandis* are often unable to molt or pupate (as in figs. 1F–1H). Emerging adults are often deformed or die half emerged. Larvae may be chalky in appearance because of the production of many spores

that replace the fat-body tissue. Insects infected as adults show a decreased reproductive rate and longevity. With other neogregarine species, only the more general debilitating effects common to protozoan infections give any indication that disease is present in reared stock. In these cases, periodic samples of susceptible insect tissues must be taken to monitor for this pathogen. Neogregarine species are known with both narrow and wide host ranges. Some genera of the Eugregarinida (*Stictospora* spp. and others) form spores that are contained in large hemocoelic cysts (figs. 2B and 2C); these spores are similar to neogregarine spores. See also Weiser (1969, figs. 273–276).

Microscopic examination.—As with the coccidia, the developmental stages are easily Giemsa-stained, but the spores are stain resistant (unless pretreated with acid). Again, phase optics will allow one to distinguish the ovoid-to-spindle-shaped spores in wet mounts from the coccidian spore type (Weiser 1969, figs. 277–312).

Viruses

Viruses are submicroscopic, obligate, intracellular pathogens that influence the host cell to replicate their DNA or RNA (depending on the virus type) rather than the cellular nucleic acids of the host; they require the living host cell for their reproduction. At the most primitive level of life, these microbes cannot grow, since they contain only their nucleic acid, a protein coat, and a small number of enzymes. They are more resistant than several other microbial groups to some environmental conditions, including freezing; and, once present in an insectary, they may spread widely in the air as aerosols, on dust particles, and commonly on adult insect scales, hairs, and bristles where cleanliness is not scrupulously maintained. The question of their spread in populations of insects by transovarial transmission and “latency” is still not completely resolved after years of study (Longworth 1973); many earlier reports have been contradicted by later work, particularly in Japan, showing no such occult (or host-genome-connected benign) virus presence in insects reared under germ-free conditions.

Viruses pathogenic to insects are classified according to the criteria established for other animal and plant viruses. These include the type of nucleic acid in the virion or mature virus particle, the particle morphology, the subunit symmetry of the protein coat, the presence or absence of an envelope surrounding the particle, and the particle size and its degree of resistance to certain chemicals (Fenner 1976, Matthews 1979).

The natural classification of insect viruses divides them firstly into groups containing RNA and DNA and second-

ly into those that either are embedded in protein-matrix bodies when mature or are naked when mature. Of the several viral families, groups, and genera present in invertebrates (Fenner 1976, p. 100), I will consider only the RNA-matrix-embedded Reoviridae (cytoplasmic polyhedrosis viruses), the RNA-naked Picornaviridae and similar viruses, the DNA-matrix-embedded Baculoviridae (granulosis viruses and nuclear polyhedrosis viruses), and the DNA-matrix-embedded Poxviridae (the genus *Entomopoxvirus*). These four virus groups are the most likely to cause insectary difficulties or are known to persist in insect populations over a long period at a low level (enzootic). Most can be expected to cause insidious continual losses and to debilitate stock insects as do the microsporidia. A few, such as the *Antheraea* virus and certain polyhedrosis viruses, can be epizootic or catastrophic in insectaries. Although individual viruses cannot be seen with a light microscope, the protein-matrix bodies that some of them are embedded in can be seen and are here treated as diagnostic indicators.

Reoviridae (cytoplasmic polyhedrosis viruses)

Disease cycle.—Sharp-cornered, distinctively shaped cytoplasmic polyhedra (or virus-containing matrix bodies) from contaminated feces or ruptured cadavers infect lepidopteran, neuropteran, dipteran, or hymenopter larvæ when ingested. They dissolve in the midgut, releasing virus particles that invade and replicate in the midgut epithelium and later often in the hindgut and foregut cells. Restriction of these viruses to the cytoplasm of the gut cells of infected larvæ is the prime diagnostic characteristic of this virus group. As the infected midgut cells lyse, polyhedra may be regurgitated or voided in the feces.

Symptoms and signs.—The growth of infected larvæ is slowed because of reduced feeding. Infected small larvæ can eventually be recognized by their extreme retardation compared with normal larvæ. The head may become disproportionally large, and an abnormal white or yellowish coloration may be seen ventrally in some larvæ where the cuticle is more transparent. This coloration is due entirely to the whitened or yellowish, obviously infected midgut, which can be seen in open dissection; transparent infected specimens such as mosquito larvæ can be recognized without dissection (figs. 2D and 2E). Adults can transmit the virus to their offspring through surface contamination of the eggs, and infected laboratory colonies may show reduced reproduction rates. These viruses are widely cross infectious throughout the Lepidoptera. Only one each is known from the Neuroptera and Hymenoptera and only a few from the Diptera.

Microscopic examination.—Wet smears or Giemsa-stained

smears of infected gut tissue should reveal many small (1–15 μ m), clear, crystalline matrix bodies (polyhedra) when viewed by light microscopy. The polyhedra usually appear rounded since they are generally too small for their sharp-cornered, characteristic, polyhedral shape to be discerned except by electron microscopy (Maramorosch 1977). The polyhedra remain clear and unstained in Giemsa-stained smears, fat globules stain purple to red, and whole cells become differentially colored in nuclei (pink) and cytoplasm (purple). Some other crystals, such as ureate concentrations, will also be stain negative but are usually much larger than the polyhedra. Use of a 1N HCl acid hydrolysis step before staining allows differentiation between cytoplasmic and nuclear polyhedrosis polyhedra; both may occur in the midguts of hymenopterans and dipterans (see the Baculovirus "Microscopic Examination" section).

Picornaviridae and similar viruses

The Picornaviridae virus group is here taken to include the known insect picornaviruses that are nominally placed in Enterovirus "genus 2" (Longworth 1978). This genus includes the cricket paralysis viruses and *Drosophila* C virus and probably acute bee paralysis virus; sacbrood virus of honey bees, *Apis mellifera* Linnaeus; and the *Mansonia uniformis* (Theobald), a mosquito, virus. There are also some similar but incompletely characterized RNA-virus groups near the Picornaviridae: (1) the Nudaurelia B group, including the (lepidopteran) viruses from *Nudaurelia cytherea capensis* Stoll, *Antheraea eucalypti* Scott, *Philosamia cynthia* Drury, *Hyalophora cecropia* Linnaeus, and others; (2) the "Group 5" viruses, including *Drosophila* P and A viruses, Kashmir bee virus, flacherie (silkworm) virus, *Gonometa podocarp*i Aurivillius (lasiocampid) virus, and others; (3) the ovoid viruses, including chronic bee paralysis virus and *Drosophila* RS virus. Longworth (1978) characterized these newer RNA-virus groups and others that are probably less important to insect culturists. He emphasized that we have only discovered a few examples of what will probably become a quite large and varied series of invertebrate-virus groups.

Disease cycle.—Since the picornaviruses are not a discrete group, it is not surprising that they have different pathologies. Most are dysentery viruses that infect the guts of larvæ; they are usually spread through the contaminated regurgitate and often copious anal discharges. Sacbrood virus is usually found in larval fat body and musculature, but it has recently been found to infect young adult worker bees and drones through contaminated food. It is now known to be dispersed through many tissues, including the salivary glands in the adult bees and in the larvæ. Adults, therefore, may feed the virus to larvæ in contaminated pollen (Gochnauer 1978).

The chronic and acute bee paralysis viruses have been found only in adult honey bees or in bumblebees. Acute paralysis virus is carried benignly by adults in nerve tissues (brains) but causes a lethal fat-body infection when transferred between bees by injection. The chronic paralysis virus is a more obvious debilitating and lethal nervous-tissue disease of adults that is transmissible by feeding (Gochnauer 1978).

Symptoms and signs.—The largely lepidopteran dysentery viruses may be highly lethal (the *A. eucalypti* virus kills infected larvae in 12 hours) or debilitating and slowly lethal. Silkworms, *Bombyx mori* (Linnaeus), infected with flacherie virus are noted as weak, flabby, feeble, and sluggish larvae that become soft and putrified in the later disease stages (Vaughn 1974). Kellen and Hoffmann (1980) found that the slower chronic stunt virus (fig. 2F) replicates only in the granular hemocyte cells (adipocytes) of the navel orangeworm, *Amyelois transitella* (Walker). Bee larvae infected with sacbrood virus appear normal until the final molt, when they fail to shed their last skin. This becomes a transparent sac around the pupal integument (Bailey 1973, fig. 22-1). Such larvae die in their brood chambers. Likewise, the tetragonal virus of mosquitoes and biting midges (Clark and O'Grady 1975) causes a similar condition; the larval thorax wall becomes an inflated sac surrounding the internal tissues (Kellen et al. 1963, figs. 4 and 5). This virus, originally thought to be a cytoplasmic polyhedrosis virus (Kellen et al. 1966), was named for the large tetragonal masses of virus particles concreted in the hypodermal and imaginal bud cells (Kellen et al. 1963, fig. 3). Moribund larvae become sluggish and abnormally curved (fig. 2G). The thoracic cuticle develops hard, shiny black spots (fig. 2H). The infection progresses slowly, killing larvae by the fourth instar. Infected insects usually remain attached to the water surface after death.

Chronic bee paralysis virus (fig. 2I) causes distension of the abdomen, leg and wing trembling, failure to fly, and leg paralysis; death occurs in about 1 week (reflecting the nervous-tissue infection). (See also Bailey 1973, fig. 22-1, and Gochnauer 1978 for further descriptions and photographs of this condition). Other paralysis viruses (of crickets, etc.) may cause general (lack of coordination) or specific (rigid, extended hind legs) paralytic symptoms that reflect infections of nervous tissues either alone or with muscular-tissue involvement (see Scotti et al. 1980). The host ranges of these other paralysis viruses are usually fairly narrow; but a few viruses, like the cricket paralysis group, can infect several hosts, even crossing ordinal lines (Lepidoptera to Orthoptera).

Microscopic examination.—These small viruses occur naked in the cytoplasm of those certain tissues often involved in symptom expression. Their confirmation can be

done only by electron microscopic study of likely tissues taken from living infected hosts. Representative photographs of these viruses have been published by Smith (1976) and by Maramorosch (1977).

Baculoviridae (nuclear polyhedrosis viruses and granulosis viruses)

Only one described virus family, the Baculoviridae, multiplies only in invertebrates. Within this family, the genus *Baculovirus* (Fenner 1976) contains both the nuclear polyhedrosis viruses (subgroup A) and the granulosis viruses (subgroup B). The invasive virions of these subgroups are embedded or occluded either individually (in granulosis capsules) or in multiples (in polyhedrosis polyhedra) in paracrystalline, protein-matrix bodies that protect the virions when they are apart from host tissue. The capsules of the granulosis viruses are usually ellipsoid; the polyhedra of the nuclear polyhedrosis viruses vary from blunt-cornered or rounded polyhedral to the rarer crescentic and fusiform, known from only two viruses infecting certain dipterans.

Another subgroup, C (representing at least one additional viral genus), has recently been added to accommodate the nonembedded or nonoccluded baculoviruses. While the subgroup A- and B-viruses of the genus *Baculovirus* are generally lethal viruses having matrix bodies visible under a light microscope, the subgroup C-viruses have a broader range of host association and can be seen only under the electron microscope.

Disease cycle.—Transmission occurs when polyhedra, capsules, or naked viruses (subgroup C only) from regurgitate or anal discharge (sawflies) or from ruptured cadavers (all host groups) are ingested by susceptible larvae; they then dissolve, releasing their virus particles. These particles penetrate the midgut epithelium and undergo one replication cycle in the epithelial cell nuclei. Virus particles are rarely occluded in the few uncommon polyhedra that are formed in these cells in the Lepidoptera. Most progeny of the gut-cell cycle move out of the gut cells into hemolymph, where they are transported to the susceptible polyhedra-producing tissues. In the Lepidoptera, polyhedra are produced in various combinations of several tissues, including the hypodermis, tracheal matrix, blood, and fat body but also to some extent the glandular tissues and reproductive organs. In the Diptera, there are fewer types of polyhedra-producing tissues. In the Tipulidae, only the fat body produces polyhedra. In the Culicidae, only the midgut epithelium produces polyhedra (earlier reports of polyhedra in other culicid tissues involved a picornavirus). In the Hymenoptera (sawflies), there is the most restriction; polyhedra are produced only in the midgut epithelium. Among the granulosis viruses, the fat body is the major

capsule-producing tissue; in many lepidopteran hosts it is the only capsule-producing tissue. But granulosis capsules are sometimes also formed in the tracheal-matrix and hypodermal tissues. The complete *Baculovirus* replication cycle was first delineated accurately by Adams in 1975 when she discovered the nature of the hemocoelic virus form (Adams et al. 1977).

Symptoms and signs.—Depending on the dosage and age of the infected larvae, signs and symptoms may occur sooner (in young larvae) or quite late (in older larvae). Or, as in some situations with the slower viruses, there may be no apparent signs or symptoms, and seemingly healthy adults may be formed that lay virus-contaminated eggs. In these cases, signs and symptoms occur quite soon in the next generation, when many young larvae die because they have ingested virus from their own contaminated egg shells as they chewed their way out. Usual signs and symptoms include a reduction in feeding and a general sluggishness. The hemolymph may become milky white rather than clear. If the hypodermal cells become infected, the whole larva may change color, becoming somewhat whitened or yellowed (figs. 3A–3C). In some cases, the abnormal white coloration may be subtle (fig. 3D) and may be accompanied by some localized swelling (fig. 3E). In some cases, an open dissection may reveal presumptively infected tissues when compared to a normal insect (figs. 3F and 3G). Earlier infections or granulosis infections (fig. 3H) may more closely resemble the normal insect. In either case, the dissection should be followed up with a microscopic study of the presumptively infected tissues. With the sawfly polyhedroses (restricted to the gut), larvae become yellowish, their guts becoming an opaque milky white. When disturbed, they produce a white regurgitate rather than the normal clear yellow or greenish regurgitate. Eventually, they exude a brown fluid from the anus, and this glues them to the substrate where they turn brown and finally black. Diseased sawfly larvae change their behavior, becoming solitary rather than gregarious and wandering randomly rather than together (Bailey 1973). Infected individuals of some species climb to the highest point available before dying. Moribund larvae become flaccid and rupture easily. After death they often hang head downwards by their posterior prolegs; the integument becomes quite fragile as the contents liquify (fig. 3I). Granulosis cadavers are less fragile than polyhedrosis cadavers, particularly when only the fat body is producing capsules. Granuloses may progress as fast as polyhedroses; but, in general they are more prolonged, extending from a few days to a month or more if death occurs before pupation.

While the granuloses may be generally restricted to the Lepidoptera (there is only one reported from a sawfly), the nuclear polyhedroses have been reported from the

Coleoptera, Diptera, Hymenoptera, and Lepidoptera. Nonoccluded (subgroup C) baculoviruses have been reported as lethal agents in the Coleoptera (spreading venereally between infected adults but lethal to larvae) and in two acarine mites. Other possible members of subgroup C apparently do not affect their hosts in the Coleoptera, Homoptera, Hymenoptera, and Araneida. Certain members of subgroup C, infecting the calyx epithelium of adult braconid and ichneumonid wasps (Stoltz and Vinson 1979, Vinson et al. 1979) seem to be mutualistic with their parasitoid hosts, either suppressing the encapsulation reaction of the lepidopteran host or otherwise influencing the host response.

Within each virus taxonomic group, the experimentally investigated host range may be quite narrow to fairly broad. For example, the granuloses are generally restricted to a single host genus or a single family. In the Lepidoptera, some polyhedroses are apparently as narrowly restricted as the granuloses, while others can easily cross family lines. Nonlepidopteran polyhedroses seem to be at least family restricted.

Microscopic examination.—Wet smears or Giemsa-stained smears of infected tissue (fat body, tracheal matrix, hypodermis, or midgut) should reveal 1–15 μm , blunt-cornered or rounded polyhedra in the nuclei of infected cells (see Steinhaus 1949, chapter 11, for wet-mount photographs of polyhedra and granulosis capsules). These will be Giemsa-stain negative (as are the cytoplasmic polyhedrosis polyhedra). An acid pretreatment, however, (1.0 N HCl for 10–20 minutes) will render nuclear polyhedrosis polyhedra stainable (gray to blue-purple with a 60–90 minute stain time); cytoplasmic polyhedrosis polyhedra will remain clear under these conditions since the stain is more readily removed by the final rinse in buffer (see the “Disease Recognition” section). The gut-restricted, culicid nuclear polyhedrosis virus will concrete fusiform polyhedra (which are confined to the nuclei). Similar bodies are formed in the cytoplasm of other tissues in some entomopoxvirus infections. Tipulid polyhedrosis polyhedra are crescent-shaped. Granulosis capsules are quite small, being just at the limit of resolution of the light microscope (up to 0.5 by 0.2 μm). When an infected cell is ruptured while being viewed in wet mount, the tiny capsules will stream out in a dense cloud.

Poxviridae (*Entomopoxvirus*)

The genus *Entomopoxvirus* is apparently restricted to the invertebrates (Fenner 1976); all isolates known so far are from the Insecta. It has been divided into three subgenera on the basis of virus morphology (and coincidentally by host range): subgenus A has unilateral concave virion cores and infects nine known species of Scarabaeoidea; subgenus B has cylindrical-to-rectangular

virion cores and infects the Lepidoptera and Orthoptera; and subgenus C has biconcave cushion-shaped (dumbbell-shaped in cross section) virion cores and infects chironomid and culicid Diptera (see Kurstak and Garzon 1977).

Disease cycle.—In the Lepidoptera and the Orthoptera, the virus replication cycle usually takes about 10–30 days; but, in the Scarabaeid Coleoptera, where the host life cycle may take 1–2 years, the virus replication cycle may take 5–10 months or longer depending on the age of the larva when infected. Since these viruses are largely confined to the fat body, usually only ruptured cadavers, cannibalism, or predators can furnish the matrix bodies containing infectious viruses (or free viruses from the hemocoel if parasitoids are involved), which usually initiate the infection. In the Lepidoptera, however, moribund infected larvae often regurgitate or defecate fluid containing virus matrix bodies. When ingested, these bodies (here called spheroids) dissolve in the midgut, and the viruses penetrate the midgut epithelial cell cytoplasm. It is not yet known what mechanisms are involved in the passage of the entomopoxviruses through the gut cell. The virus particles eventually reach the target tissues, where final replication and occlusion (in spheroidal matrix bodies) completes the replication cycle. The cycle has been described by several authors, including Granados (1973), Kurstak and Garzon (1977), and Vaughn (1974).

Symptoms and signs.—Infected insects exhibit a general sluggishness, high mortality, and prolonged developmental stages. In the Lepidoptera, death is often preceded by paralysis of the gut and by regurgitation or defecation of fluid containing infectious spheroids. In the Scarabaeoidia (larvae having transparent cuticles), an externally visible white-spotted or mottled appearance often develops in the dorsoposterior area of heavily infected individuals; this appearance results from the enlargement of infected fat-body tissue and possibly from infection of the hypodermis (fig. 5A; see also Goodwin and Roberts 1975, figs. 1–5). In some transparent dipteran larvae, the infection of the fat body can be readily seen externally (fig. 5B), which greatly aids in distinguishing infections. In the Orthoptera (*Acrididae*), heavily infected insects become quite distended with obviously protruding cervical membranes due to swelling of the spheroid-packed fat body. Some lepidopterans also show this effect, becoming larger and longer than normal larvae before succumbing (fig. 5C). Seen in open dissection, a fat body infected with *Entomopoxvirus* (fig. 5D) may be a flat chalky white or grayish in infections lacking spindles. Where spindles are present, the fat body takes on a reflective, foamy appearance (Goodwin and Roberts 1975, fig. 6). The entomopoxviruses are restricted to cross infectivity within the family of origin so far as is known (Kurstak and Garzon 1977).

Of interest to insect culturists is a recent report describing the density-dependent action of an entomopoxvirus in a laboratory colony of chironomid midges (Harkrider and Hall 1979).

Microscopic examination.—Of the several entomopoxviruses isolated from four insect orders, almost all produce spheroidal virus matrix bodies only in the fat body or in the fat body and hemocytes. Only two are more general in the tissues attacked. One is restricted to the hemocytes alone. The virus-containing spheroid is parallel to the polyhedron of the nuclear and cytoplasmic polyhedrosis viruses; it is a paracrystalline protein matrix dissolvable at certain high pH ranges with a combination of dilute alkali plus a reducing compound such as dimercaptopropanol, sodium thioglycolate, or cysteine. (Polyhedra from the other virus groups do not require the addition of reducing agents for dissolution.) The size, form, and distribution of the spheroids depend on the virus species. They range in shape from nearly spherical (coleopteran hosts) to nearly regular ellipsoidal (lepidopteran hosts). Some unusual spheroid shapes have also been noted, as in the case of the *Demodema bonariensis* Bruch virus (rounded subcubical) and the *Melanoplus sanguinipes* (Fabricius) virus (ellipsoidal in sagittal section but almost square in cross section). The *Figulus sublaevis* Beauvois (Lucanidae), *Chironomus luridus* Strencke, and *Camptochironomus tentans* (Fabricius) virus spheroids may be more irregularly globose to rounded polyhedral (Goodwin and Filshie 1975). So each virus type usually has a strong degree of uniformity of spheroid shape, while there is a wide range of shapes among the different virus types or species.

The diagnostic utility of the spheroids (which are easily overlooked in wet-mount preparations of lightly infected insects—Moore and Milner 1973), is supplemented in some cases by the presence of more distinctive virus-related fusiform or spindle-shaped accessory or inclusion bodies. The diseases of the *Entomopoxvirus* group were in fact originally called the spindle viruses until it was found that the spindles never contain the viruses and are not always present. They are absent in the known entomopoxviruses infecting the Diptera and the Orthoptera but are present in some but not all of the viruses infecting the Scarabaeoidia and the Lepidoptera. Spindles range in size from the tiny microspindles, occluded with virus particles in some lepidopteran spheroids, to 25- μ m macrospindles that are larger than many of the known spheroids. The pointed spindles are readily distinguishable by shape in wet-mount smears when viewed with phase optics (Goodwin and Filshie 1969). They stain readily with Giemsa (usually light to dark blue); the mature spheroids remain unstained. Partly formed “immature” spheroids of some of these viruses stain gray or gray-brown with Giemsa alone (Goodwin

and Roberts 1975). Prolonged (1 hour or more) Giemsa staining of certain unresponsive types (*Aphodius tasmaniae* Hope virus) results in a green staining reaction of both spindles and spheroids. But, if this long Giemsa staining is preceded by a 2-hour acid hydrolysis treatment with 1N HCl, the spindles will stain a pale gray-brown; the spheroids will stain medium to dark blue and reveal inner reddish spots that may relate to the often-seen refractile fissures or the contained virus particles (Goodwin and Roberts 1975).

A diagnostic stain for the differentiation of both *Entomopoxvirus* spheroids and spindles was described by Moore and Milner (1973). It involves the use of concentrated lactophenol cotton blue for staining fresh wet smears; the rapid result is that both spheroids and spindles are colored intensely blue, which distinguishes them from fat globules, uric acid crystals, and other types of occluded viruses. To prepare this stain (which is also used as a mounting medium and stain for fungi), combine the following: phenol crystals (100 g), lactic acid (USP 85%; 80 ml), glycerol (159 ml), and distilled water (100 ml); add 0.5% cotton blue (anilin blue, C.I. 42755, or methyl blue). This preparation will keep at room temperature indefinitely (Poinar and Thomas 1978). Add the stain directly to the dried smear and cap with a cover slip. The stain is also a mounting medium and will intensify with time.

Rickettsiales and Chlamydiales (see p. 117)

The rickettsiae are a group of lower micro-organisms that includes both obligate parasites and commensals of arthropods, though the group has some features common to the bacteria. Rickettsiae have a cellular structure including a cell wall, they contain both RNA and DNA, and they contain active metabolic enzyme systems. The insect pathogens in the rickettsial tribe Wolbachiae were thought to be restricted to arthropods, but some have more recently been shown to be infectious to warm-blooded vertebrates. Of the four known genera in the Wolbachiae, one, the intracellularly occurring *Wolbachia*, contains types that show no particular tissue tropisms and that may be transmitted through the eggs from infected females. These have been isolated from mosquitoes, lice, ticks, and mites. It is possible that some rickettsiae in this genus may be symbiotes. A second genus, *Rickettsoides*, grows (epicellularly) on the gut epithelium of the host (some lice, fleas, and parasitic flies), but they are apparently harmless to their hosts. Two genera, *Enterella* and *Rickettsiella*, contain species pathogenic to insects (see Krieg 1963 and 1971b).

A series of chlamydiae showing affinities to both *Enterella* and *Rickettsiella* infections, but that manifest themselves only as stunting agents for a wide variety of hosts, has been found by J. R. Adams (unpublished data). These

chlamydiae have been isolated from hypodermis tissue (sometimes causing black spotting of the integument) and occasionally also from the midgut. One chlamydia can also be transovarially transmitted in *Trichoplusia ni* (Hübner)—embryonal hypodermal cells contain chlamydiae. Laboratory-reared species found infected include the German cockroach, *Blattella germanica* (Linnaeus), and the following lepidopterans: the range caterpillar, *Hemileuca oliviae* Cockerell; the redbacked cutworm, *Euxoa orchogaster* (Guenée); the southwestern corn borer, *Diatraea grandiosella* (Dyar); the tobacco budworm, *Heliothis virescens* (Fabricius); the bollworm, *Heliothis zea* (Boddie); the cabbage looper, *Trichoplusia ni* (Hübner); the European corn borer, *Ostrinia nubilalis* (Hübner); the almond moth, *Ephestia cautella* (Walker); the pink bollworm; the saltmarsh caterpillar, *Estigmene acrea* (Drury); and the tobacco hornworm, *Manduca sexta* (Linnaeus). Chlamydiae were also found in nerve tissue of infected field-collected species including the satin moth, *Leucoma salicis* (Linnaeus); the melon aphid, *Aphis gossypii* Glover; the cereal leaf beetle, *Oulema melanopus* (Linnaeus); the beetle *Coccinella septempunctata* (Linnaeus); the sugarbeet root maggot, *Tetanops myopaeformis* (Roder); and the alfalfa weevil parasite, *Bathyplectes* sp.

Enterella

Disease cycle.—The rickettsiae of this genus are usually associated with the host gut epithelium, but they grow intracellularly rather than epicellularly. This genus has few described members. Some are apparently insignificant in their pathogenic effects on hosts. Others are lethal through their destruction of the midgut epithelium (as, for example, *Enterella stethorae*). Transmission is through ingestion. The rickettsiae are probably spread through regurgitate and fecal contamination of the environment and by rupture of cadavers. Such rickettsiae are known so far only from the coccinellid coleopteran *Stethorus* and the culicid dipterans *Culex*, *Anopheles*, and *Aedes* and from one lepidopteran.

Symptoms and signs.—The *Enterella* isolated from *Hyalophora cecropia* (Linnaeus) and *Samia cynthia* (Drury) (Lepidoptera: Saturniidae) causes a lethal, perhaps toxic, dysentery. Infected larvae show a decreased rate of growth and eventually stop feeding entirely. The body weight decreases 1–2 weeks before death. Diarrhea precedes regurgitation, and both symptoms are accompanied by violent muscular contractions of the body. Moribund larvae are generally flaccid, but some die in a contracted position. The course of the disease is about 14 days in larvae (Entwistle and Robertson 1968).

Microscopic examination.—The lepidopteran *Enterella* described above was found primarily in the midgut but

was also isolated from the fat body and the hypodermis of infected insects. Normal staining procedures were unsatisfactory in revealing the rickettsiae; but, if smears or sections were pretreated for 3–5 minutes with 1N HCl at 60° C and then stained overnight in dilute Giemsa solution (pH 7.5–7.8), results were excellent. The cell cytoplasm stained light blue, the nuclei, dense violet, and the intracellular rickettsial organisms and gut bacteria, red. Both *Enterella* and *Rickettsiella* are often visible in chains (Entwistle and Robertson 1968), which distinguishes them from granulosis virus capsules that are also quite tiny but never occur in chains.

Rickettsiella

Disease cycle.—*Rickettsiella* are known to survive for more than 3 years in the soil. They also show some resistance to heat, chemicals, antibiotics, and radiation (Krieg 1971b). They are spread through ingestion from ruptured cadavers and cannibalism. Long-lived coleopteran larvae of the Scarabaeidae show signs or symptoms after about 2–3 months, but they may not die until 6 months after infection (at 20° C). The rickettsiae penetrate the midgut epithelium and chiefly infect the fat body, but there may be some involvement of the ovaries, ganglia, tracheal matrix, malpighian tubules, musculature, and hypodermis, depending on the rickettsial species.

Symptoms and signs.—Infected larvae become sluggish as the disease develops, probably due to the involvement of nervous tissue later in the infection. Acrididae, Blattellidae (fig. 4A), Gryllidae, Tenebrionidae, and Carabidae (fig. 4B) infected by *Rickettsiella* often show a swelling of the abdomen as the fat body, packed with rickettsiae, becomes enlarged within. This abdominal swelling is usually apparent because of the exposure of the intersegmental membranes that are normally folded beneath the segmental sclerites. Discolorations of infected larvae are common in these infections, particularly among hosts with transparent cuticles. Infected tipulids and chironomids become abnormally whitened (as in fig. 1A) as the proliferating rickettsiae are released from the fat-body cells and blood cells into the hemolymph. A similar chalky whiteness due to the same cause is apparent in certain infected scarabaeid larvae (*Melolontha* spp., Europe; *Anoplognathus* spp., Australia; and *Costelytra* spp. and *Odontria* spp., New Zealand). *Rickettsiella popilliae* will color infected Japanese beetle, *Popillia japonica* Newman, larvae bluish ventrally so that externally it resembles an Iridovirus infection. I have observed several Australian *Rickettsiella* infections in scarabaeid larvae and am reporting them here for the first time: (1) an infection in larvae of the blackheaded pasture cockchafer, *Aphodius tasmaniae* Hope, that causes a scattered black spotting of the integument probably caused by hypodermal cell

death (fig. 4C); (2) an infection in larvae of the tableland pasture scarab, *Antitrogus* (formerly *Rhopaea*) *morbillosus* (Blackburn), that colors the legs, head, anal area, and spiracles a deep blue black (figs. 4D–4F); (3) an infection in larvae of the cocksfoot grub, *Rhopaea verreauxi* Blanchard, resulting in a green and brown mottling of the entire integumental surface and a progressive darkening as the insects become moribund; and (4) an infection in several species of melolonthine larvae in which lobes of infected fat body swell due to rickettsial growth within and then break free and float around in the hemolymph (figs. 4G–4H). In all these infections, typical rickettsiae were isolated from the fat body, stained with Giemsa as smears, and then morphologically confirmed as rickettsiae by electron microscopy. All infected larvae showed a progressive sluggishness but no other notable symptoms.

The host range of the known *Rickettsiella* species is wide among those few that have been so investigated (Krieg 1971b). *Rickettsiella* are widely infectious between host families and perhaps also between host orders. *Rickettsiae* may take a long time to kill their hosts, and infected hosts may not have very obvious symptoms (these may be similar to the stunting syndrome in *Enterella*). Because of these factors and since they are quite resistant to destruction in the field apart from their hosts, *Rickettsiella* species may be expected to cause considerable losses before being discovered in insectaries that use frequently gathered field stock (fig. 4I).

Microscopic examination.—The *Rickettsiella* are stainable with Giemsa in smears as are other rickettsiae. Although the typical chains of mature rickettsiae (also typical of mature *Enterella*) may not be seen until quite late in the infection, there are more obvious earlier microscopic signs of this genus. Various sized intracellular rickettsiae-filled vacuoles (called RFV or NR bodies in the earlier literature) retain their structure and stain violet to deep purple with Giemsa in fat-body squash or smear preparations (see Krieg 1963, fig. 4). Eventually, these vacuoles release mature rickettsial progeny. Usually, variously shaped, large, stain-negative crystals are also associated (inside and outside) with the rickettsiae-filled vacuoles in such smears. The rickettsiae-filled vacuoles are more useful as presumptive diagnostic indicators of *Rickettsiella* than the rickettsiae themselves since they are more readily stained and are present earlier and over a longer period during such infections.

Bacteria

The bacteria are minute (0.2–5.0 μ m), unicellular plantlike organisms that differ from higher plants in that they lack chlorophyll and do not contain organelles. They are classified by shape into four main groups: rod-shaped bacilli, spherical cocci, comma-shaped spirilla, and branched or-

ganisms of the actinomycetes. Many are motile by means of flagella.

Insect relationships of bacteria

Bacteria are widely distributed throughout the environment and so, like the fungi, are common contaminants of artificial insect diets and insectary stock insects. In the insectary environment, and particularly where artificial diets are used, several bacterial genera may colonize the diet and then the guts of stock insects, resulting in continuous stock losses (McLaughlin and Sikorowski 1978) that may be attributed to other causes. Some of the bacterial genera and species implicated in such insectary mortality have been listed among the normal microflora of a number of insects, particularly *Streptococcus* spp. (Jarosz 1979), but also *Aerobacter* (*Enterobacter*) spp. (Nunez et al. 1968), *Escherichia* spp., *Erwinia* spp., *Proteus* spp., *Micrococcus* spp., *Alcaligenes* spp., and *Flavobacterium* spp. (Pristavko 1966). Many investigators have listed these and other bacteria "from diseased and dead larvae" and demonstrated larval mortality by feeding them to laboratory insects (see several studies cited by Lipa and Wiland 1972). It is apparent from these and other conflicting studies that many of the listed "microflora" are only fortuitous contaminants. Those that cause diseases in insectaries reflect as much the ability of some bacteria to outcompete others as the ability of some to actively invade insects.

There are many complex interactions between bacteria and other microbes as well as between insects and bacteria (Brooks 1963). Some "established" symbiotic relationships, such as those occurring in fruit flies, especially those reported by Boush and Coppel (1974) for the olive fruit fly, *Dacus oleae* (Gmelin), have come into question when studied more closely (Yamvrias et al. 1970). Recent work with the walnut husk fly, *Rhagoletis completa* Cresson, has demonstrated a loose bacterial symbiotic relationship that may vary between fly strains or species and that may change as the fly diet changes, either in the field or in the insectary (Tsiropoulos 1976). The fact that *D. oleae* strains adapted to artificial diets cannot survive on olives (Fytizas and Mazomenos 1971) may indicate either a genetically deficient fly strain or the loss of a very specific and morphologically distinctive bacterial symbiote that has not yet been cultured apart from the fly (Poinar et al. 1975).

Although some bacterial genera (*Serratia marcescens*, *Pseudomonas* spp., and others) have repeatedly caused field epizootics in sawflies and are recorded as true invasive pathogens in certain insects (Grimont et al. 1979a, Poinar et al. 1979), such bacteria are more often classifiable in other insect hosts as noninvasive facultative pathogens. To cause disease, these depend on

factors such as high humidity or temperature stress (Goodwin 1968, Greany et al. 1977, Habib 1978), wounding, or tearing of the hindgut or foregut during molting or pupation (Goodwin 1968). Some bacteria related to the facultative pathogens—for example, *Serratia ficaria* in the fig wasp, *Blastophaga psenes* (Linnaeus), and others (Grimont et al. 1979a, 1979b)—may be either insect commensals or loosely associated symbiotes. For example, *Streptococcus* spp., while apparently beneficial to some insect species (Jarosz 1979), may be primary invasive pathogens in others (Doane 1971); in this case, the species *Streptococcus faecalis* was responsible for both effects.

Management of insectary bacteria

Depending on the insect species, specific strains of *S. faecalis* may be considered for use in prophylactic gut flora, perhaps in association with other bacterial species (Goodwin 1968) such as the *Erwinias* and other plant-rotting bacteria, particularly in the insectary rearing of phytophagous insects (Martin and Mundt 1972). Also, such action may be simpler and preferable to the maintenance of aseptic rearing conditions in preventing the activities of the facultatively pathogenic bacteria. While germicide additions to artificial insect diets are now commonplace, the use of antibiotics is to be discouraged in general. Such use encourages further unnecessary selection of resistant strains of bacteria and often merely masks an unfavorable insectary environmental condition that should be corrected. Instead, further study should be given to the addition of leaf extracts from usual host plants (Goodwin 1968) that may contain bacteriostatic or bactericidal flavonoids or terpenes. Such extracts may contain factors that interact with the insect gut to produce protective compounds, such as active caffeic acid, which has been isolated from the guts of leaf-fed silkworms; caffeic acid has been shown to protect silkworms against pathogenic *S. faecalis* isolates and other bacteria (Koike et al. 1979). Again, more naturally microbe-florated gut microenvironments may be related to improved survival and competitiveness in colonized parasitoids or genetically altered stock that is to be used for field release.

Symptoms and signs

Since some bacteria will be present even within insects reared in aseptic or clean environments, they must be expected in decomposed insects, whatever the cause of death. Therefore, careful evaluation must follow the detection and isolation of bacteria from insectary stock. Dead insects killed by facultative bacterial pathogens in the insectary often reflect transient unfavorable conditions such as adverse humidity and/or temperature. Moreover, large numbers of insects, even among those reared

individually but in the same location, will often contain similar bacteria, these being local ambient air or surface contaminants. Insects infected by *S. marcescens* are usually pinkish to red when infected by pigmented strains; but many nonpigmented strains exist that are also pathogenic (Grimont et al. 1979a). Cadavers of insects infected with *Pseudomonas* spp. may appear greenish and exude a heavy, sweet odor. Other bacteria also give off characteristic odors when present in cadavers in near monocultures.

Insects infected with bacteria become progressively more sluggish before death, but other more definitive symptoms rarely occur. At death or shortly thereafter, the usually septicemic growth of bacteria in the hemocoel will have largely decomposed most of the internal tissues. Further discussion of symptoms and signs of the spore-forming and nonsporeforming primary bacterial pathogens can be found in Dutky (1963, milky diseases), Bucher (1963, nonsporeformers), Heimpel and Angus (1963, sporeformers), Faust (1974, bacterial diseases, general), and Shimanuki (1978, bacterial diseases of bees).

Microscopic examination

A wet-mount smear may reveal a uniform type of bacteria and its motility, if it is present as a septicemic agent in the hemocoel. If a Giemsa smear from the infected insect confirms that only bacteria of a relatively or completely uniform morphology are present (rods of a certain size, cocci, or spirillae), then a Gram stain (Poinar and Thomas 1978, pp. 187-188) may confirm the presence of a uniform type (Gram positive or negative). If further identification of the bacterium is warranted, it may be isolated by use of the techniques described by Poinar and Thomas (1978, pp. 60-77 and 167-180). Precise identifications can be obtained from contract laboratories. If mixed types are present in the cadavers, one should attempt to isolate bacteria either from the moribund insects or from

those that are living but diseased so as to segregate possible pathogens from the many saprophytic forms that usually luxuriate in cadavers.

Fungi

The fungi are heterotrophic micro-organisms with chitinized cell walls; they are typically nonmotile, though motile stages (zoospores) may be present. Most of the entomogenous fungi contain hyphae (fungal hairlike strands developing from a germinating spore) that, grouped together, constitute a mycelium (fungal mat or mass). Reproduction is mainly by sexual or asexual spores. Asexual spores are borne in taxonomically characteristic sporangia (as sporangiospores) or on characteristically shaped special hyphae or conidiophores (as conidiospores).

Many fungi, like bacteria, are opportunists; that is they are facultative parasites or saprophytes rather than primary pathogens. Often in the insectary, saprophytic fungi will be found growing on the surface of dead insects as well as on the food and frass in rearing chambers. Such fungi usually do not solidify, or mummify, host insects as many of the entomogenous pathogens do. Entomogenous fungi occur among four classes: the Zygomycetes, including *Entomophthora*, *Massospora*, and others; the Chytridiomycetes, including *Coelomomyces*; the Ascomycetes (sacfungi), including *Ascosphaera*, *Cordyceps*, and others; the Basidiomycetes (club fungi), including *Septobasidium*; and the Deuteromycetes (Fungi imperfecti, sexual stages unknown), including *Aspergillus*, *Beauveria*, *Hirsutella*, *Isaria*, *Metarrhizium*, *Nomuraea* (*Spicaria*), *Paecilomyces*, and others. For a more complete generic listing, see Roberts and Yendol (1971, table 1); and, for the associations between genera of fungi and insect host taxons (including references), see the listing by Bell (1974, table 1) and key 2, which follows.

Key 2.—Key to common insect fungi³

1. Thallus attached to chitinous gut lining or exoskeleton by a secreted holdfast or a specialized holdfast cell 2
- 1'. Thallus without a holdfast, intercellular or intracellular 3
- 2(1). Thallus mycelial, not organized into a sporocarp, spores one-celled, mostly commensals or symbionts of aquatic Diptera, Ephemeroptera, and certain Coleoptera *Trichomycetes* (Lichtwardt 1973, illus.,⁴ keys;⁵ Moss 1979, illus., descrip.⁶)
- 2'(1). Thallus reduced, forming a cellular, perithecioid ascocarp; often hairlike or setose; ascospores two-celled; obligate ectoparasites *Laboulbeniomyces* (fig. 6A; Benjamin 1973, illus., keys; Tavares 1979, illus., keys)
- 3(1'). Hyphae lacking or scarce, septate, reproduction by yeastlike cells *Candida* and related genera (Lodder 1970, illus., keys, descrip.)
- 3'(1'). Hyphae abundant, septate or unseptate 4
- 4(3'). Mycelium unseptate (septa formed only during reproduction) and fragmenting into uninucleate or multinucleate hyphal bodies (*Mastigomycota*) 5
- 4'(3'). Mycelium septate throughout the life cycle (*Ascomycotina* and *Deuteromycotina*) 8
- 5(4). Hosts aquatic, usually in larvae of Culicidae; zoospores present *Coelomomyces* Keilin (Couch 1945, illus., keys, descrip.; McNitt and Couch 1977; Whisler 1979; Burges 1981, illus., descrip., see keys to *Coelomomyces* and *Lagenidiales*)
- 5'(4). Hosts terrestrial, zoospores absent 6
- 6(5'). Spores produced outside the host, often between intersegmental folds *Entomophthora* Fresenius (fig. 6B; Macleod and Müller-Kögler 1973, illus., keys, descrip.; Macleod et al. 1976, illus., keys, descrip.; Waterhouse 1973)
- 6'(5'). Spores produced in the host 7
- 7(6'). Spores aggregated on a palisade of conidiophores and discharged through an abdominal hole on muscoid diptera *Strongwellsea* Batko & Weiser (Humber 1976, illus., keys, descrip.)
- 7'(6'). Spores surrounded by hyphae and released on disintegration of the host abdomen, restricted to Cicadidae *Massospora* Peck (Soper 1974, illus., keys, descrip.)
- 8(4'). Spores within asci (ascospores) formed in perithecia (subclass *Ascomycotina*) 9
- 8'(4'). Spores on conidiophores that are solitary, in pycnidia, acervuli or on coremia (subclass *Deuteromycotina*) 13
- 9(8). Asci scattered throughout the ascocarp 10
- 9'(8'). Asci arranged in a hymenium 11
- 10(9). Ascocarps formed within a stroma, ascospores septate, usually restricted to scale insects *Myriangium* Montagne & Berkeley (Miller 1940, illus., keys, descrip.; von Arx 1963)
- 10'(9). Ascocarps (erronously referred to as cysts by some) formed superficially on a white, cottony mycelium; ascospores unseptate and grouped as spore balls; on Apoidea *Ascospaera* Olive & Spiltoir (fig. 6C; Gilliam 1978; Skou 1972, illus., descrip.)
- 11(9'). Ascospores two- to five-celled, ellipsoid or fusoid *Nectria* (Fries) Fries
- 11'(9'). Ascospores many-celled, filiform or elongate fusiform 12
- 12(11'). Hosts mummified; perithecia borne within a stalked, light-colored, stromatic head; ascospores fragmenting at septa *Cordyceps* Link (Mains 1958, illus., keys, descrip.; McEwen 1963)
- 12'(11'). Hosts not mummified, perithecia borne on a superficial, effused-to-pulvinate, dark-colored stroma; ascospores not fragmenting *Hypocrella* Saccardo and *Podonectria* Peck (Petch 1921)

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⁴illus.—The reference has illustrations of the taxa below the rank discussed here.

⁵keys—The reference has keys to the taxa below the rank discussed here.

⁶descrip.—The reference has descriptions of the taxa below the rank discussed here.

13(8'). Conidiophores in flask-shaped pycnidia (Sphaeropsidales), pycnidia immersed in bright-colored stromata, conidia one-celled, hyaline, sexual stages in the genera <i>Hypocrella</i> and <i>Stereocrea</i> H. & P. Sydow	14
(Mains 1959)	
13'(8'). Conidiophores not as above	14
14(13'). Conidia of two kinds: (1) Macroconidia—slightly bowed and often with chlamydospores; (2) Microconidia—one-celled	15
(fig. 6F; Booth 1971, illus., keys, descrip.; Madelin 1963)	
14'(13'). Conidia of one kind, neither bent nor septate	15
15(14'). Sporogenous cells formed on globose-to-clavate heads or swellings of the conidiophore	16
15'(14'). Sporogenous cells not formed on a swelling of the conidiophore	17
16(15). Coremia absent, conidia globose to subglobose, in definite chains	17
(fig. 6E; Madelin 1966; Raper and Fennell 1965, illus., keys, descrip.)	
16'(15). Coremia usually present, conidia fusoid to ellipsoid, solitary or in chains, confined to spiders (ascigerous stage <i>Torrubiella</i>)	17
(Mains 1950a)	
17(15'). Sporogenous cells formed on synnemata	18
(figs. 6L, 6M)	
17'(15'). Sporogenous cell usually formed on solitary hyphae	23
18(17). Sporogenous cells arranged in a hymenium	19
18'(17). Sporogenous cells usually not forming a hymenium	20
19(18). Sporogenous cells obtuse, conidia solitary and without mucous	20
(fig. 6H; Mains 1950b, illus., descrip.)	
19'(18). Sporogenous cells usually pointed, sometimes sharply so; conidia catenulate, with or without mucous	20
(figs. 6I and 6J; Mains 1960b, illus., descrip.)	
20(18'). Conidiogenous cells not elongated, conidia dry	21
(DeHoog 1972, illus., keys, descrip.)	
20'(18'). Conidiogenous cells elongated and usually tapering to a point, conidia mucilaginous	21
21(20'). Conidiogenous cells usually enlarged at the base, conidia alone or in groups of two or more in droplets	21
(fig. 6K; Mains 1951, illus., descrip.)	
21'(20'). Conidiogenous cells not enlarged at the base, conidia held in a conspicuous mucilaginous ball	22
22(21'). Sclerotia present, spore balls not disintegrating readily	22
(fig. 6M; Mains 1951, illus., descrip.)	
22'(21'). Sclerotia absent, spore balls disintegrating readily	22
(fig. 6L; Mains 1951, illus., descrip.)	
23(17'). Conidia aggregated in slime at the tips of conidiophores	23
(fig. 6N; Ganhao 1956)	
23'(17'). Conidia dry or powdery	24
24(23'). Conidia borne in chains	25
24'(23'). Conidia borne singly	28
25(24). Hosts mummified; conidiophores formed in an olive-green velvety mound; conidia basipetal, cylindrical	28
(fig. 6P; Gams and Rozsypal 1973, illus., descrip.)	
25'(24). Host usually not mummified, conidia ellipsoid or globose	26
26(25'). Conidiogenous cells clustered on penicillate, nondivergent conidiophores	26
(fig. 6G; Raper and Thom, 1949, illus., keys, descrip.)	
26'(25'). Conidiogenous cells clustered or solitary along divergent, often verticillate conidiophores	27
27(26'). Conidia pale green or light purple (green or purple in mass), broad—ellipsoid or cylindrical	27
(fig. 6D; Getzin 1961; Sampson 1974, illus., descrip.)	
27'(26'). Conidia hyaline, ellipsoid to fusiform	27
(For <i>P. farinosus</i> Gray, an entomogenous sp., see Sampson 1974)	
28(24'). Conidia borne on minute sterigmata, conidiogenous area often zigzag	28
(fig. 6O; DeHoog 1972, illus., keys, descrip.)	
28'(24'). Conidia borne on depressions on the conidiophores	28
(see Petch 1938 for entomogenous species)	

Disease cycle

Most entomogenous fungi initiate infection with a germinating spore (conidium) that adheres to and penetrates the cuticle of the insect. The invasive hypha grows, enters the host tissues, and ramifies through the hemocoel. As a result, the fungus becomes distributed throughout the host's hemocoel, filling it with hyphae (mycelial mass). After death, the solidified host may dry and become hard (mummification). On incubation of the mummified cadaver in a moist environment, emergence hyphae grow out through the insect's integument and produce spores (usually conidia) on the external surface of the host (see Steinhaus 1949, chapter 10; Weiser 1969, figs. 180, 181, 190C, 203, 213, 216-219, 222, 242-250 and Poinar and Thomas 1978, figs. 3-41). These spores are dispersed by forcible ejection (as in fig. 5E) or by wind to contact further hosts. In certain aquatic forms (*Coelomomyces* spp.), rupture of the cadaver releases asexual sporangia (Weiser 1969, figs. 159-166) that later release zoospores infectious to alternate hosts (*Copepoda*). A similar (sexual) cycle occurs in the alternate host, and the zoospores produced there are infectious to the original mosquito or other dipteran host species (Whisler 1979). Both *Coelomomyces* and similar aquatic fungi in the Lagenidiales and Saprolegniales were described more recently by Bland, Couch, and Newell in chapter 8 of Burges (1981). The general fungus disease cycle is described in more detail by Roberts and Yendol (1971) and by MacLeod (1963), Madelin (1963), and Bell (1974), who also discuss the environmental conditions favoring fungus infections.

Symptoms and signs

Larvae may show black spots at the sites of spore penetration as an early disease sign. There may be nervous activity and restlessness followed by sluggishness and a cessation of feeding before death. Often, color changes (to brown, fig. 5F, or to pink, red, purple, or yellow, fig. 5G) are apparent before or after death. When the emergence hyphae grow out of the solidified cadaver and form conidia, the resulting macroscopic appearance may allow a presumptive diagnosis (figs. 5E, 5F, 5I). *Cordyceps* infections are particularly characteristic (McEwen 1963) but are unlikely to occur in insectaries. Various fungus infections that are characteristic in appearance have been demonstrated in illustrations by Steinhaus (1949), Weiser (1969), and Poinar and Thomas (1978). More extensively described symptoms and signs appear in Steinhaus (1949), Couch and Umphlett (1963), McEwen (1963), MacLeod (1963), Madelin (1963), Bell (1974), and Lipa (1975). Fungi in bees are described by Gilliam (1978). Earlier generic keys to some entomogenous fungi were presented by Weiser and Briggs (1971) and Poinar and Thomas (1978). More recent keys to the Deuteromy-

cetes; Entomophthorales; and aquatic fungi in the Lagenidiales, Saprolegniales, and *Coelomomyces* are given in Burges (1981).

Microscopic examination

Incubation of the suspect solidified cadaver (terrestrial, not aquatic, hosts) in a moist chamber, will cause outgrowth of the characteristic conidiophores or spore-bearing emergence hyphae (figs. 5G-5I). These can be pulled off with forceps and mounted on a slide with Guegen's solution (Poinar and Thomas 1978) or lactophenol cotton blue (see above in the section on microscopic examination of *Entomopoxvirus*) to render the characteristic morphology more easily visible. Then, using key 2 and the microscopic illustrations in figure 6 (as well as the photographs presented in Weiser 1969, Poinar and Thomas 1978, and Burges 1981), one can make a presumptive generic identification. Photographs or drawings of unusual morphological types and cultures provided on suitable agars (Poinar and Thomas 1978) would aid specialists in determining unusual types accurately if a species identification is necessary.

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Figures 1-6 follow on pages 118-129.

Note Added in Proof

A recent serological analysis by J. R. Adams has placed the stunting "chlamidia" described here closer to the Rickettsiales than to the Chlamydiales. Further work may place them in a new rickettsial tribe or other subgrouping.

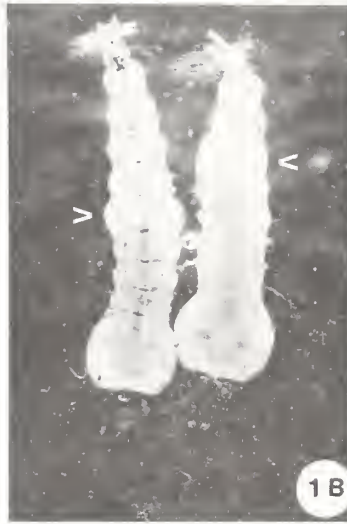


Figure 1

A. Chironomid larvae. Fat body infected with a microsporidian, *Telomyxa* sp. See fig. 5B for appearance of normal larva. (Photograph courtesy of the Gulf Coast Mosquito Research Unit, U.S. Agricultural Research Service, Lake Charles, La.)

B. *Anopheles* sp. (Culicidae) larvae. Fat body infected with a microsporidian, *Nosema* sp.; arrows indicate swollen, diseased abdominal segments. (Photograph courtesy of the Gulf Coast Mosquito Research Unit, U.S. Agricultural Research Service, Lake Charles, La.)

C. *Aedes sollicitans* (Culicidae) larvae. Left larva infected with a microsporidian, *Thelohania* sp.; right larva normal. (Photograph courtesy of the Gulf Coast Mosquito Research Unit, U.S. Agricultural Research Service, Lake Charles, La.)

D. *Culiseta incidens* (Culicidae) larvae. Left larva, with oenocytes infected by a microsporidian, *Thelohania* sp.; right larva normal. (Photograph courtesy of W. R. Kellen, Stored-Product Insects Research Laboratory, U.S. Agricultural Research Service, Fresno, Calif.)

E. *Amyelois transitella* (Pyralidae) larvae infected with the microsporidian *Nosema invadens*, showing characteristic black spotting of the integument. (Photograph courtesy of W. R. Kellen, Stored-Product Insects Research Laboratory, U.S. Agricultural Research Service, Fresno, Calif.)

F. *Amyelois transitella* (Pyralidae) larvae. Normal-sized larvae above; smaller larvae below stunted by the microsporidian *Nosema invadens*. (Photograph courtesy of W. R. Kellen, Stored-Product Insects Research Laboratory, U.S. Agricultural Research Service, Fresno, Calif.)

G. *Amyelois transitella* (Pyralidae) pupae, normal. (Photograph courtesy of W. R. Kellen, Stored-Product Insects Research Laboratory, U.S. Agricultural Research Service, Fresno, Calif.)

H. *Amyelois transitella* (Pyralidae) pupae, malformed by the microsporidian *Pleistophora* sp. (Photograph courtesy of W. R. Kellen, Stored-Product Insects Research Laboratory, U.S. Agricultural Research Service, Fresno, Calif.)

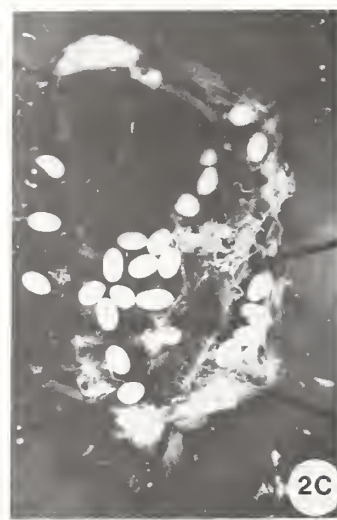
I. *Sericesthis geminata* (Scarabaeidae) larvae. Normal larva below; larva above infected with the coccidian *Adelina sericesthis* early stages, causing translucent clearing of dorsal tissues indicated by arrow.



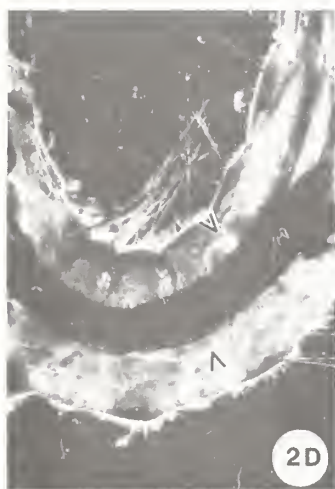
2A



2B



2C



2D



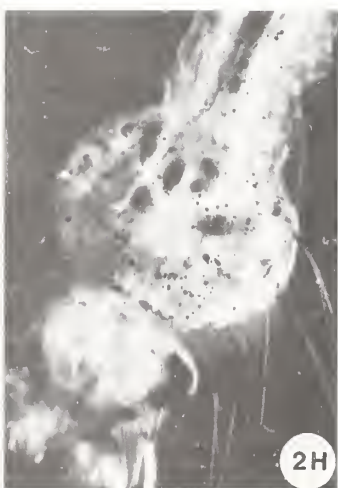
2E



2F



2G



2H



2I

Figure 2.

A. *Sericesthis geminata* (Scarabaeidae) larva showing the characteristic brown mottling caused by oocyst formation in a late infection with the coccidian *Adelina sericesthis*.

B. *Rhopaea verreauxi* (Scarabaeidae) larva containing hemocoelic cysts of a gregarine *Stictospora* sp.; external view.

C. *Rhopaea verreauxi* (Scarabaeidae) larva containing hemocoelic cysts of a gregarine *Stictospora* sp.; open dissection.

D. *Culex salinarius* (Culicidae) larva. External view by transmitted light showing a cytoplasmic polyhedrosis virus (Reovirus) infection of the midgut. Arrows denote swollen midgut portions containing polyhedra. (Photograph courtesy of T. B. Clark, Insect Pathology Laboratory, U.S. Agricultural Research Service, Beltsville, Md.)

E. *Aedes sollicitans* (Culicidae) larvae. Left insect with a cytoplasmic polyhedrosis virus (Reovirus) infection of the midgut; right insect with a nuclear polyhedrosis virus (Baculovirus) infection of the midgut. (Photograph courtesy of T. B. Clark, Insect Pathology Laboratory, U.S. Agricultural Research Service, Beltsville, Md.)

F. *Amyelois transitella* (Pyralidae) larvae. Top two larvae normal; small larvae below showing stunting due to infection with chronic stunt virus (a picornavirus). (Photograph courtesy of W. R. Kellen, Stored-Product Insects Research Laboratory, U.S. Agricultural Research Service, Fresno, Calif.)

G. *Culex tarsalis* (Culicidae) larvae. Bottom insect apparently normal; middle insect moribund, showing abnormal curvature and distended thorax characteristic of the tetragonal virus (picornavirus) infection. (Photograph courtesy of W. R. Kellen, Stored-Product Insects Research Laboratory, U.S. Agricultural Research Service, Fresno, Calif.)

H. *Culex salinarius* (Culicidae) larva showing cuticular black spotting characteristic of infection with the tetragonal virus (picornavirus). (Photograph courtesy of the Gulf Coast Mosquito Research Laboratory, U.S. Agricultural Research Service, Lake Charles, La.)

I. *Apis mellifera* (Apidae) adult showing the swollen abdomen characteristic of infection with the chronic bee paralysis virus (picornavirus). (Photograph courtesy of A. J. Gibbs, Department of Developmental Biology, Australian National University, Canberra, A.C.T., Australia.)

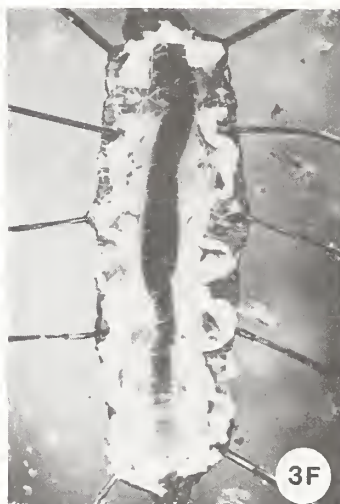
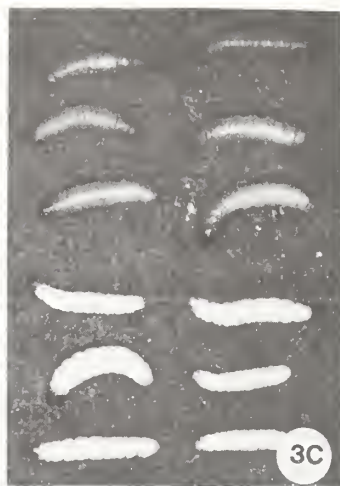


Figure 3

A. *Pieris rapae* (Pieridae) larvae. Top two larvae are dark green and normal; larva below shows pale yellow-green coloration caused by a granulosis virus (Baculovirus) infection. (Photograph courtesy of R. E. Teakle, Department of Primary Industries, Indooroopilly Brisbane, Queensland, Australia.)

B. *Euxoa auxiliaris* (Noctuidae) larvae. Left larva retarded and with abnormal pale coloration due to a granulosis virus (Baculovirus) infection; right larva normal. (Photograph courtesy of G. R. Sutter, Northern Grain Insects Research Laboratory, U.S. Agricultural Research Service, Brookings, S.D.)

C. *Plodia interpunctella* (Pyrilidae) larvae. Lower six whitened larvae infected with a granulosis virus (Baculovirus); upper six brown larvae normal. (Photograph courtesy of W. R. Kellen, Stored-Product Insects Research Laboratory, U.S. Agricultural Research Service, Fresno, Calif.)

D. *Wiseana* sp. (Hepialidae) larvae, ventral aspect. Left larva normal; right larva shows swollen and whitened intersegmental areas caused by a nuclear polyhedrosis virus (Baculovirus) infection. (Photograph courtesy of S. G. Moore, Microbiology Department, Medical School, Dunedin, New Zealand.)

E. *Wiseana* sp. larvae, ventral aspect close up. Normal larva on the left; larva on the right infected with nuclear polyhedrosis virus and shows localized swelling due to distended internal tissues. (Photograph courtesy of S. G. Moore, Microbiology Department, Medical School, Dunedin, New Zealand.)

F. *Wiseana* sp. (Hepialidae) normal larva in open dissection. (Photograph courtesy of S. G. Moore, Microbiology Department, Medical School, Dunedin, New Zealand.)

G. *Wiseana* sp. (Hepialidae) larva infected with nuclear polyhedrosis virus (Baculovirus); open dissection; note disruption of fat body. (Photograph courtesy of S. G. Moore, Microbiology Department, Medical School, Dunedin, New Zealand.)

H. *Wiseana* sp. (Hepialidae) larva infected with granulosis virus (Baculovirus); open dissection; with fat body somewhat more proliferated than in normal larva of figure 3F. (Photograph courtesy of S. G. Moore, Dunedin, New Zealand.)

I. *Trichoplusia ni* (Noctuidae) post mortem. Larvae infected with nuclear polyhedrosis viruses (Baculoviruses) characteristically assume this posture, hanging by their posterior prolegs when they die. (Photograph courtesy of A. M. Heimpel, Insect Pathology Laboratory, U.S. Agricultural Research Service, Beltsville, Md.)



4A



4B



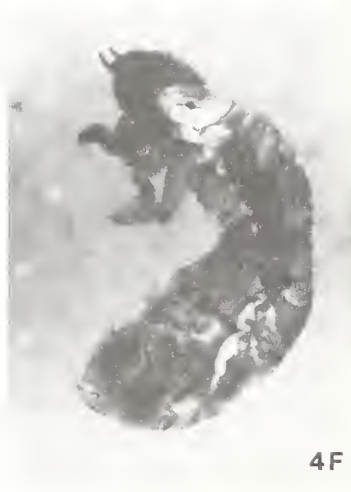
4C



4D



4E



4F



4G



4H



4I

Figure 4

A. *Blatta orientalis* (Blattidae) cockroaches. Middle insect normal; top and bottom insects have swollen abdomens revealing paler intersegmental membranes caused by infection with a *Rickettsiella* sp. (Photograph courtesy of A. M. Huger, Institut für biologische Schadlingsbekämpfung, Darmstadt, West Germany.)

B. Carabid beetle showing a swollen abdomen caused by infection with a *Rickettsiella* sp. (Photograph courtesy of A. M. Huger, Institut für biologische Schadlingsbekämpfung, Darmstadt, West Germany.)

C. *Aphodius tasmaniae* (Scarabaeidae) larva. Integumental black spotting caused by an infection with a *Rickettsiella* sp.

D. *Antitrogus (Rhopaea) morbillosus* (Scarabaeidae) larva showing abnormal blue-black spiracles caused by an early infection with a *Rickettsiella* sp.

E. *Antitrogus (Rhopaea) morbillosus* (Scarabaeidae) larva showing a later infection than that shown in figure 4D. The blue-black discoloration caused by *Rickettsiella* sp. has progressed to include the head, legs, and anal portions.

F. *Antitrogus (Rhopaea) morbillosus* (Scarabaeidae) larva. Same insect as that in figure 4E but post mortem, showing that the characteristic discoloration caused by a *Rickettsiella* sp. infection is retained for some time after death.

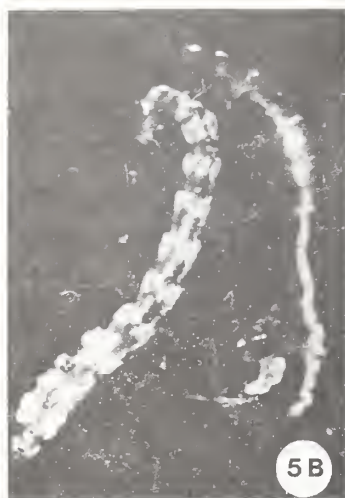
G. *Antitrogus (Rhopaea) morbillosus* (Scarabaeidae) larva showing an external view of a *Rickettsiella* sp. infection that causes swollen infected lobes of fat body to break off from the normal tissue and float around loosely in the hemolymph; the arrow indicates the most prominent loose floating lobes.

H. *Antitrogus (Rhopaea) morbillosus* (Scarabaeidae) larva, in open dissection, showing the same insect as that in figure 4G. The fat-body lobes infected with *Rickettsiella* sp. disperse in the dissection water when the diseased larva is opened.

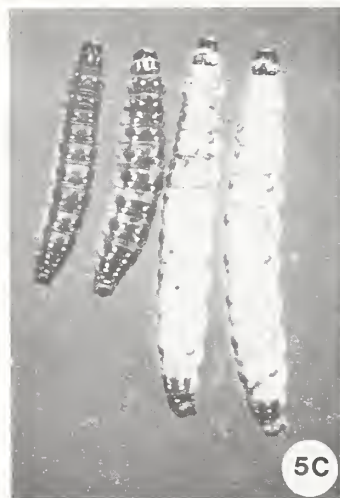
I. *Diabrotica speciosa vigans* (Chrysomelidae) adults. Normal dark green insect below. Top insect pale green with a yellow-green abdomen caused by infection with a *Rickettsiella* sp. (Photograph courtesy of J. R. Adams, Insect Pathology Laboratory, U.S. Agricultural Research Service, Beltsville, Md.)



5A



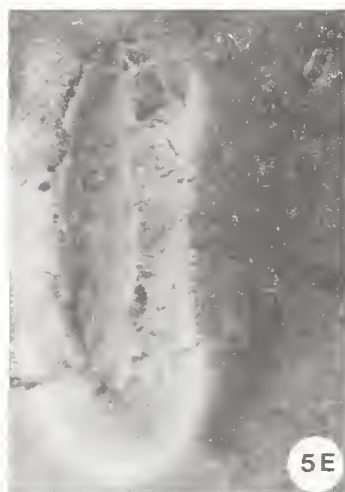
5B



5C



5D



5E



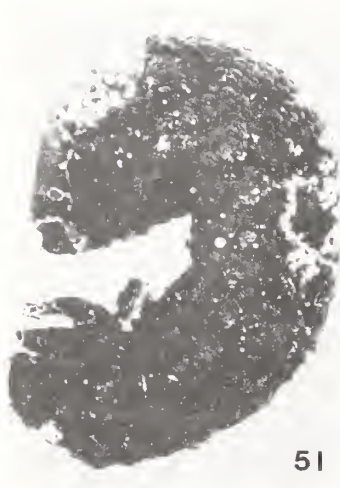
5F



5G



5H



5I

Figure 5

A. *Othnonius batesi* (Scarabaeidae) larva showing a late infection with an *Entomopoxvirus*. Arrow indicates abnormally whitened hypodermal tissue that is transparent in the normal larva; infected larvae turn progressively dull white throughout (compare with the generally normal appearance of the closely related larva in fig. 4G).

B. Chironomid larvae. Left larva shows abnormally opaque-white segmental fat body caused by an *Entomopoxvirus* infection; right larva shows normal transparent aspect. (Photograph courtesy of the Gulf Coast Mosquito Research Unit, U.S. Agricultural Research Service, Lake Charles, La.)

C. *Choristoneura fumiferana* (Tortricidae) larvae. Left two larvae normal; right two larvae show the extreme distension and lightening characteristic of this *Entomopoxvirus* infection in late instar larvae. (Photograph courtesy of F. T. Bird, Forest Pest Management Institute, Sault Ste. Marie, Ontario, Canada.)

D. *Wiseana* sp. (Hepialidae) larva in open dissection, showing some increase in fat-body tissue caused by an *Entomopoxvirus* infection but also by the spherical cysts formed by a parallel infection of the fat body with a coccidian. (Photograph courtesy of S. G. Moore, Microbiology Department, Medical School, Dunedin, New Zealand.)

E. *Plutella maculipennis* (Yponomeutidae) larvae. Left larval cadaver showing outgrowth of an *Entomophthora* sp. fungus through the integument and forcible ejection of sticky, infective spores, which form a ring around the cadaver at the limits of their range. Normal larva on the right. (Photograph courtesy of R. E. Teakle, Department of Primary Industries, Indooroopilly Brisbane, Queensland, Australia.)

F. *Rhopaea verreauxi* (Scarabaeidae) mummified, brown larval cadaver showing early outgrowth of the infecting *Hirsutella* sp. fungus on incubation in a moist chamber for 5-7 days.

G. *Anoplognathus* sp. (Scarabaeidae) mummified (solidified), yellow larval cadaver as recovered from the field. Compare this fungus infection with the same insect in figures 5H and 5I.

H. *Anoplognathus* sp. (Scarabaeidae) mummified larval cadaver after 3 days incubation in a moist chamber; note early outgrowth of white hyphae of infecting fungus and early (green) fruiting bodies (conidiospores) in small patches laterally on each segmental spiracle of the host.

I. *Anoplognathus* sp. (Scarabaeidae). Same larval cadaver as in 5G and 5H but after 7 days incubation in a moist chamber, surface of larva covered with the characteristic (green) conidiospores of the infecting green muscardine fungus *Metarrhizium anisopliae*.



**Figure 6—Camera Lucida Drawings
of Fungi Viewed With a Microscope**

A, Laboulbenia. Left, 2 one-septate ascospores with mucilaginous sheath; right, thallus with appendages and a basal holdfast. *B, Entomophthora.* Conidiophores and conidia. *C, Ascosphaera.* An ascocarp with several globose asci and ellipsoid ascospores. *D, Nomuraea.* Conidiogenous cells and conidia. *E, Aspergillus.* A conidiophore with globose head, sporogenous cells, and conidia. (Adapted from Raper and Fennel 1965.) *F, Fusarium.* Macroconidia and microconidia. *G, Penicillium.* A branched conidiophore, conidiogenous cells, and conidia. *H-J, Hymenostilbe, Akanthomyces, and Insecticola,* respectively. A hymenium of sporogenous cells and conidia (adapted from Mains 1950a, 1951). *K, Hirsutella.* Sporogenous cells and conidia with mucilage. *L and M, Tilachlidium and Synnematum,* respectively. Synnemata with conidiogenous cells and mucilaginous spore balls. *N, Cephalosporium.* Mucilaginous spore balls on solitary conidiophores. *O, Beauveria.* Conidiogenous cell, zigzag deticules, and conidia. *P, Metarrhizium.* Clustered conidiophores and conidia. (Figure prepared by L. R. Batra, Mycology Laboratory, Plant Protection Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md. 20705.)

Micro-organisms as Contaminants and Pathogens in Insect Rearing

By Martin Shapiro¹

Introduction

Although historically insect pathology has concentrated on the honey bee, *Apis mellifera* Linnaeus, and the silkworm, *Bombyx mori* (Linnaeus), because of their economic importance (see, for example, Bassi 1835, Pasteur 1870, Steinhaus 1956), this paper will discuss how micro-organisms may affect the rearing of many species and what measures may be used to prevent or minimize their impact (see also Steinhaus 1953, Helms and Raun 1971, and McLaughlin 1971).

Micro-organisms may have little effect on insects, or they may destroy an entire colony (Steinhaus 1953, 1968), depending on the micro-organism involved and the measures used to minimize or eliminate it. For example, disease was a major obstacle to successful rearing of the corn earworm, *Heliothis zea* (Boddie), in 1967-69 (Sparks and Harrell 1976). And Raulston and Lingren (1972) stated that viral and protozoan diseases and diet contamination by bacteria and fungi were often responsible for insect-rearing failures. They concluded that failure to control microbial contaminants and pathogens could offset the value of new rearing techniques.

The most common microbial contaminants encountered in insect cultures are *Aspergillus* spp. fungi (see, for example, Leonard and Doane 1966, Chawla et al. 1967, Hensley and Hammond 1968, Kishaba et al. 1968, Fidet 1972, Griffin and Lindig 1973, and Ludemann et al. 1979). These fungi affect insect-rearing programs in several ways. For example, *Aspergillus* growth covering the rearing medium has prevented young pink bollworm, *Pectinophora gossypiella* (Saunders), larvae from feeding (Ouye 1962). But *Aspergillus flavus* Link and *A. niger* van Tieghem growing as saprophytes on the feces of the cabbage looper, *Trichoplusia ni* (Hübner), do not harm it, because contamination occurs mainly during prepupation and pupation (Ignoffo 1964, 1966a). In one program, which was rearing the codling moth, *Laspeyresia pomonella* (Linnaeus), *A. niger* contamination occurred mainly at the end of the larval feeding period (Howell 1971). The larvae left the areas where, in some instances, the fungal hyphae had penetrated the diet. Then some

larvae died from starvation, and those crawling through the fungal growth became covered with conidia; some of these insects died, presumably from asphyxiation. The incidence of *Aspergillus* contamination increased in the rearing area from year to year and reduced moth yield from 125.5 moths per tray to 64.6 per tray.

Many other microbes can harm laboratory-reared insects. For example, Doane (1969) found that hatching gypsy moth, *Lymantria dispar* (Linnaeus), larvae are often contaminated by bacteria and fungi. In general, these micro-organisms are not pathogenic, but their growth on artificial diet adversely affects larval development. Baumhover et al. (1977) reported that, in one case, microbial contamination of the diet caused losses of 80%-85% of a tobacco hornworm, *Manduca sexta* (Linnaeus), culture despite preventive measures. Gast (1966) reported that contamination from *Pseudomonas* spp. in boll weevil, *Anthonomus grandis grandis* Boheman, cultures reduces adult yield and increases adult mortality.

Viruses are another group of micro-organisms that can harm laboratory-reared insects. For example, the only larval disease observed in codling moth rearing is a granulosis virus. In one program, this virus disease was present initially in only a few insects, but two epizootics occurred within a year and severely reduced insect production (Howell 1971). Similarly, examination of diseased and dead codling moth larvae in a Russian rearing program showed that 55.3% were infected with a granulosis virus (Pristavko et al. 1971). In another program, the incidence of granulosis increased among Indian meal moth, *Plodia interpunctella* (Hübner), larvae in successive generations and caused many deaths (Spitler 1970). Early attempts to rear the cabbage looper failed because of repeated virus epizootics (McEwen and Hervey 1960), and Henneberry and Kishaba (1966) concluded that nuclear polyhedrosis virus was a major impediment in large-scale rearing of the cabbage looper.

Protozoa, mainly microsporidia, can present serious problems in the rearing of many insects, for example, the honey bee; the European corn borer, *Ostrinia nubilalis* Hübner; and corn earworm. Laboratory cultures of the alfalfa weevil, *Hypera postica* (Gyllenhal), often suffer from high levels of infection caused by *Nosema* spp. (Hsiao and Hsiao 1973). Nosemosis has also affected rearing of the boll weevil; Gast (1966) reported a dramatic increase in occurrence of *Nosema* spp. over eight generations in the laboratory; this was correlated with reduced

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egg production and eventual destruction of the colony. He also reported a similar sequence of events when the boll weevil colony was infected by another protozoan, *Mattesia grandis* McLaughlin. A third protozoan, similar to *Glugea gasti* McLaughlin, occurred in 0%-100% of the weevils examined, without causing apparent symptoms. During epizootics, some reduction in oviposition occurred among infected females (Flint et al. 1972). In one program, adult European corn borers infected by the protozoan *Perezia pyraustae* Paillot laid fewer eggs, had a lower percentage of hatch, and had a shorter longevity than controls (Lewis et al. 1971). In another program, the cinnabar moth, *Tyria jacobaeae* (Linnaeus), which is used for biological control of the tansy ragwort, *Senecio jacobaea* L., was heavily infected by a microsporidian. The disease caused high mortality among the larvae, leading Bucher and Harris (1961) to conclude that it may play a major role in the failure of mass-reared lepidopterans to control weeds. *Nosema* infections have been reported in colonized anopheline mosquitoes by Fox and Weiser (1959), Vavra and Undeen (1970), and Hazard and Lofgren (1971). But, Hazard (1971) concluded that few epizootics occurred in colonies of anopheline species native to the United States. In fact, he traced the origin of the disease to colonies of *Anopheles* at the London School of Hygiene and Tropical Medicine that were originally collected from Africa and Asia.

Effect of the Rearing Environment on Microbial Contaminants

Movement of insects from the field to the laboratory often stimulates disease and microbial contaminants. *Serratia marcescens* (Bizzio), for example, is a common organism that often becomes pathogenic in the laboratory (Doane 1960, Wood 1961). This bacterium is typically unable to invade healthy, unwounded insects but is transmitted with other bacteria when the insects bite each other because they are crowded.

Often, bacteria contaminating insect colonies are those that are more commonly associated with man (Sikorowski 1975, Hedin et al. 1978). As early as the 1830's, Bassi (1835) recognized that silkworm muscardine, caused by the fungus *Beauveria bassiana* (Balsamo), spreads by contamination of food and of insectary personnel. And concentration of the insects into a confined space increases the chances of pathogen transmission (Gast 1966). Also, incidental fungal contamination may increase with increase of larval populations in containers (Hensley and Hammond 1968).

Steinhaus (1953) stated that controlling disease in laboratory insects requires rearing them in the best possible environmental conditions. High humidity and temperature have often been associated with increased

occurrence of diseases and contaminants. First to associate high humidity with fungal germination was Bassi (1835); more recently, Howell (1970) reported that *A. niger* develops rapidly on codling moth diet exposed to warm, stagnant air. Steinhaus (1953) observed that holding insects on diet in closed containers results in condensation caused by a temperature gradient and often increases disease occurrence. Stephens (1962) reported that mortality of greater wax moth, *Galleria mellonella* (Linnaeus), larvae caused by a bacterium, *Streptococcus faecalis* Andrewes and Harder, increases when temperature and relative humidity are high. Likewise, McLaughlin (1962) reported that mortality caused by the bacteria *Pseudomonas aeruginosa* (Schroeter), *Aerobacter aerogenes* (Kruse), and *S. marcescens* increases among armyworm, *Pseudaletia unipuncta* (Haworth), larvae with increase in temperature. And Steinhaus (1945) reported that he controlled *Serratia* and *Aerobacter* infections of the potato tuberworm, *Phthorimaea operculella* (Zeller), by regulating environmental temperature. So, proper design of the insect-rearing facility to allow control of environmental conditions is important in preventing or minimizing the occurrence of diseases and contaminants.

Effect of the Insect on Microbial Contamination

Field-collected insects are often used to start and augment colonies in the laboratory because of the availability and the need for genetic variability. They are also a major source of parasites, pathogens, and microbial contaminants. For example, Sutter et al. (1971) reported field-collected eggs of the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, to be a major source of bacteria and fungi. Likewise, Dunbar et al. (1972) reported that an entomogenous fungus, *Pasclomyces farinosus* Brown and Smith, was observed growing on gypsy moths in 88% of the collection sites. Lynch and Lewis (1978) isolated four genera of fungi and an unidentified yeast from European corn borer egg masses. Also, Lynch et al. (1976) reported isolating bacteria of six different families, and several that were unidentified, from European corn borer. Some of these bacteria reduce egg hatch, and the Bacillaceae also reduce larval establishment.

Viruses and microsporidia often occur at high rates in field populations. Collection of wild insects for starting laboratory colonies may result in introduction of these organisms. Chauthani and Claussen (1968) reported a 40% incidence of a natural virosis in Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDonnough), larvae from field-collected egg masses; and Doane (1975) reported up to 95% mortality from viruses in field-collected gypsy moth larvae. Likewise, the microsporidian *Perezia pyraustae* is

often reported from wild European corn borer populations (see, for example, Raun 1966). Spruce budworm, *Choristoneura fumiferana* (Clemens), is often naturally infected with a *Nosema* (Wilson 1974). Collecting insects from areas where they are not dense should decrease chances of colonizing diseased insects (Magnoler 1970a, 1970b), at least in the case of the gypsy moth and the Douglas-fir tussock moth (Chauthani and Claussen 1968).

Transmission of pathogens

Many pathogens are transmitted from one generation to the next either on the egg surface (transovum) or in the egg (transovarial). Hatching larvae get nuclear and cytoplasmic polyhedrosis viruses from the egg surface (Bullock et al. 1969, Sikorowski et al. 1973, Doane 1975). Gypsy moth larvae acquire virus from the egg surface or from contaminated hairs as they hatch from the egg mass (Doane 1975). Transmission through the egg was observed over 100 years ago when Pasteur (1870) noted that the protozoan *N. bombycis* could be transmitted through the egg of the silkworm. Since then, it has been demonstrated that other microsporidian parasites are passed transovarially from one generation to another (see, for example, Raun 1961; Ishihara and Fujiwara 1965; Gast 1966; Gast and Davich 1966; McLaughlin 1966, 1971; Jenkins et al. 1970; Lewis et al. 1971; and Hsiao and Hsiao 1973). Gast (1966) reported that the percentage of boll weevil eggs containing *M. grandis* varies with the percentage of diseased adult females; and, as the disease spreads from adult to adult, the number of diseased eggs increases.

Parasites are also transmitted when the spores are ingested by healthy insects (Gast 1966, Hsiao and Hsiao 1973). After the gut epithelium is infected, transmission probably occurs through contamination of feces (Bucher and Harris 1961, Lewis et al. 1971). For example, in one program, the rate of *N. bombycis* infection in silkworms was high in the first, second, and fifth larval stages, corresponding with a change in the number of larvae excreting *Nosema* spores (Ishihara and Fujiwara 1965). Another mode of transmission may be cannibalism (Gast 1966).

It should be assumed that survivors from infected cultures may have an infection; for example, Bucher and Harris (1961) found in one study that two-thirds of the apparently healthy pupae of the cinnabar moth were infected with the microsporidian *Nosema cerasivoranae* Thomson. Insect-to-insect transmission of micro-organisms also occurs with virus diseases (Jacques 1962, Henneberry and Kishaba 1966, Stewart et al. 1976) and fungus diseases (Bassi 1835, Hensley and Hammond 1968).

Diet Contamination by Micro-organisms

Contamination of the diet with fungi (Ignoffo 1966a, Leonard and Doane 1966, Chawla et al. 1967, Kishaba et al. 1968) and bacteria (Afrikian 1960, Sutter et al. 1971, Childress and Williams 1973) may be a minor inconvenience or a serious impediment to laboratory or insectary rearing. Dietary ingredients may be one source of microbial contaminants. Ignoffo (1965) isolated yeasts, molds, and bacteria from vitamins, wheat germ, casein, and water; and no microbial contaminants were isolated from potassium hydroxide, methylparaben (methyl *p*-hydroxybenzoate), choline chloride, Formalin (formaldehyde), Alphacel, agar, sucrose, or Wesson Salts (Ignoffo 1966a). I have isolated contaminants from wheat germ, casein, torula yeast, and from tap water used in gypsy moth rearing; more than 95% of the total bacterial population was isolated from the raw wheat germ (unpublished data).

Semisynthetic, artificial diets provide the nutrients essential not only for insect growth and development but also for growth and development of microbial contaminants. In many instances, contamination of the diet, mainly by fungi belonging to the genus *Aspergillus* (see, for example, Ouye 1962, Ignoffo 1966a, Chawla et al. 1967, and Kishaba et al. 1968), is the most common contamination problem. For example, Griffin et al. (1974) isolated two bacterial species and one fungus, *A. niger*, from unsterilized boll weevil diets. McLaughlin and Sikorowski (1978) isolated 16 bacterial species from insectary-reared boll weevils and tested their growability on artificial diet; only one bacterium, a *Flavobacterium*, could not grow on the diet. They further tested 35 bacterial cultures, including human pathogens. Twelve of these grew on the diet, including 5 of 15 human pathogens and 7 of 20 non-pathogens. In our laboratory at the U.S. Agricultural Research Service's Otis Methods Development Center, Otis Air National Guard Base, Mass., bacteria (*Bacillus cereus* Frankland and Frankland, *Staphylococcus aureus* Rosenbach, *Escherichia coli* (Migula) Castellani and Chalmers, *Enterobacter aerogenes* Hormaeche and Edwards, *Proteus vulgaris* Hauser) and fungi (*Aspergillus niger* van Tieghem, *Penicillium notatum* Westling, *Rhizopus* spp., *Saccharomyces cerevisiae* Meyen) have been tested for their ability to grow on a diet high in wheat germ (Bell et al. 1981) as part of a study on antimicrobials. All of the micro-organisms tested grew and developed on the insect diet (M. Shapiro, unpublished data).

The diet's pH is another factor that might favor or inhibit microbial growth and development. In one test, when the diet was adjusted to pH 5.5, the bacterial population was 1.6 million/g (at day 7); at 6.5 pH, the bacterial population increased to 290 million/g (Gifawesen et al. 1975). At the Otis Methods Development Center.

elimination of antimicrobials from the gypsy moth diet allowed contamination at pH's 4-8; antimicrobials could not inhibit microbial development at a dietary pH greater than 6.5. And larval growth was retarded at pH 7 or higher regardless of the insect diet used. Dietary pH's of 4.5-6.5 (unadjusted pH's: 5.3-5.8) were satisfactory for rearing the insect, and contamination was minimal at the acid end of the pH range (Bell et al. 1981).

Counteracting Micro-organisms in the Insect and the Diet

Sanitation and decontamination

Strict sanitation measures are needed to minimize or eliminate micro-organisms (see, for example, McEwen and Hervey 1960, Gast 1966, Henneberry and Kishaba 1966, Ignoffo 1966a, Lyon and Flake 1966, Raun 1966, Magnoler 1970a, Helms and Raun 1971, McLaughlin 1971, Sutter and Miller 1972, and Sikorowski 1975). Both rooms and equipment should be sanitized, and equipment should be autoclaved where possible. Items that cannot be autoclaved should be soaked in disinfectants such as Formalin (see, for example, Steinhaus 1953, Gast 1966, and Stewart et al. 1976) or sodium hypochlorite (see, for example, Henneberry and Kishaba 1966, Martin 1966, Odell and Rollinson 1966, and Baumhover et al. 1977). Recently, Shimanuki et al. (1969) and Tompkins and Cantwell (1975) have shown that ethylene oxide can be used to decontaminate equipment and facilities.

Chemical disinfection of the insect

Whether insects are collected from the field or reared in the laboratory, they are a source of contaminants and pathogens. To minimize or eliminate the micro-organisms, the insect should be disinfected. Any insect life stage may be disinfected, but the egg stage is most often selected.

Chemical antimicrobials.—No chemical antimicrobial is perfect, and compromises must be made with each to fit a rearing program's particular needs. The two chemicals most commonly used are sodium hypochlorite (NaOCl) and formaldehyde (in Formalin). Ignoffo and Dutky (1963) suggested that NaOCl was an ideal general disinfectant because of its broad microbicidal spectrum of activity, good solubility in water, stability in aqueous solutions, nontoxicity to humans and insects, availability, and low price. Odor and skin irritation after prolonged contact are disadvantages; and treatment of eggs with NaOCl causes partial dechoriation, resulting in susceptibility to desiccation and mechanical injury. In general, two treatments, with variations, have been used for disinfection. In the first, eggs are soaked in NaOCl and rinsed in water (see, for example, Getzin 1962). In the

second, eggs are soaked in NaOCl, treated with sodium thiosulfate to neutralize the chlorine, and rinsed in water (see, for example, Ignoffo and Dutky 1963). These methods have been used for egg disinfection (by Getzin 1962, Ignoffo and Dutky 1963, Grisdale 1968, Vasiljevic and Injac 1971, Sikorowski et al. 1975, Beckwith and Stelzer 1979, and many others), larval disinfection (see, for example, King et al. 1979), pupal disinfection (see, for example, Stone 1969), and adult disinfection (see, for example, Harein and de Las Casas 1968).

Formaldehyde is often used as an egg disinfectant (see, for example, Golanski 1961, Lyon and Flake 1966, Chauthani and Claussen 1968, Magnoler 1970a, Sikorowski 1975, Bell et al. 1981, and many others). Other chemicals that have been used as disinfectants include mercuric chloride (see, for example, Barras 1972, Singh and Fowler 1973, and Kamano 1971); quaternary ammonium salts (see, for example, Martignoni and Milstead 1960); cupric sulfate (see, for example, Nettles and Betz 1966); parahydroxymethyl-benzoate (see, for example, Cothran and Gyrisco 1966); and sorbic acid (see, for example, Karpel and Hagmann 1968). (For detailed description of these disinfectants, refer to Steinhaus 1953 and to "Microbial Contamination in Insectaries. Occurrence, Prevention, and Control," by Peter P. Sikorowski, below.)

Effects of chemical disinfectants on micro-organisms.—Protozoans are among the most difficult pathogens to remove by disinfection. In these instances, the insect is most often disinfected in the egg stage. Tyler (1962) successfully removed spores of the sporozoan *Triboliocystis garnhami* Dissanike from *Tribolium* spp. eggs by washing them in a detergent solution. And a microsporidian (*Nosema algerae* Vavra) infection in *Anopheles stephensi* Liston was reduced from over 90% to between 1% and 15% when eggs were treated with water rinses alone (Alger and Undeen 1970). But washing boll weevil eggs in a detergent (Triton X-100; 0.1%) for 5 minutes, mercuric chloride (0.06%) and NaOCl (0.8%) for 10 minutes, or Formalin (4%) for 30 minutes, failed to reduce an infection of *M. grandis* (Gast 1966).

Formalin and NaOCl have been highly effective in eliminating or suppressing fungi. Ignoffo and Dutky (1963) reduced the viability and infectivity of *B. bassiana* spores by treating them with NaOCl. Sikorowski (1975) reported that treatment of boll weevil eggs with 0.1%-0.2% NaOCl produced contamination-free eggs, and treatment of the eggs with 10% Formalin caused a 91% reduction in fungal contamination.

Formalin and NaOCl are also highly effective in reducing bacterial contamination (see, for example, Ignoffo and Dutky 1963, Sikorowski 1975, Stewart et al. 1976, and King et al. 1979). Other compounds have been used as bactericides. For example, Martignoni and Milstead

(1960) tested two quaternary compounds, Zephiran Chloride² and Hyamine 10-X,³ and reported that they had low mammalian toxicity, good bactericidal activity, and good wetting action; however, only Hyamine 10-X adequately removed bacteria from variegated cutworm, *Peridroma saucia* (Hübner), eggs. Barras (1972) tested Hyamine 10-X, mercuric chloride, White's solution,⁴ and a modified White's solution for pupal disinfection of southern pine beetle, *Dendroctonus frontalis* Zimmerman, and reported that only the modified White's solution and Hyamine 10-X effectively reduced bacterial contamination. Nettles and Betz (1966) reported controlling bacteria on boll weevil eggs by treating them with 18% cupric sulfate solution (for 35 minutes) followed by treatment in a 25% ethyl alcohol solution with 0.04% mercuric chloride.

Formaldehyde and NaOCl are used alone and in combination for control of viruses. Ignoffo (1964) reported effectively sterilizing cabbage looper eggs with NaOCl, and Vail et al. (1968) demonstrated NaOCl and formaldehyde to be effective in eliminating nuclear polyhedrosis virus. Grisdale (1968) controlled virus infection in the forest tent caterpillar, *Malacosoma disstria* Hübner, with a 0.5% NaOCl wash. Several studies report using NaOCl to disinfect field eggs of the gypsy moth (see, for example, Leonard and Doane 1966; ODell and Rollinson 1966; Doane 1967, 1969, 1975; and Smith et al. 1976) to minimize nuclear polyhedrosis virus levels.

Formaldehyde has often proven to be more effective than NaOCl as a viricidal egg disinfectant. Thompson and Steinhaus (1950) recommended the use of a 10% formaldehyde solution (for 90 minutes) as an egg disinfectant. Later, Golanski (1961) tested many chemical solutions for controlling nuclear polyhedrosis virus in silkworm cultures and reported formaldehyde as most effective. Formaldehyde was also found most effective for controlling nuclear polyhedrosis virus in Douglas-fir tussock moth by Lyon and Flake (1966) and Chauthani and Claussen (1968); cytoplasmic polyhedrosis virus in pink bollworm, by Bullock et al. (1969), Mangum et al. (1972), and Stewart et al. (1976); granulosis virus in the Indian meal moth, by Spitler (1970); and granulosis virus in *Pieris brassicae*, by David et al. (1972).

Effects of chemical disinfectants on insects.—Chemical disinfectants can harm the insect. For example, treatment of eggs with NaOCl may cause dechoriation that allows the eggs to dry out and hatch early (see for ex-

ample, Gast 1966, Vail et al. 1968, Sutter et al. 1971, and Beckwith and Stelzer 1979). In many of these cases, NaOCl was replaced with Formalin, which causes less severe dechoriation (Bullock et al. 1969). But reduced hatch after formaldehyde treatment of eggs has been reported (by Howell 1970, David et al. 1972, and 1975). Reduced egg hatch has also been reported as a side effect of treatment with other materials, such as methylparaben and sorbic acid (Greene 1970), Hyamine 10-X, and Zephiran Chloride (Sutter et al. 1971).

Heat treatment to disinfect insects

The egg stage for several species can tolerate higher temperatures than the pathogen will. Finney et al. (1947) and Allen and Brunson (1947) reported using this difference to control *Nosema* in a potato tuberworm by heating the eggs to 47° C. Likewise, immersing eggs of European corn borer in a 43.4° C water bath for 30 minutes to control *P. pyraustae* was a successful treatment for several years (Raun 1961); this treatment eventually lost its effectiveness, apparently because of selection of a heat-resistant *Nosema* (Lewis and Lynch 1970). Heat has also been used to reduce the incidence of cytoplasmic polyhedrosis virus in the tobacco budworm, *Heliothis virescens* (Fabricius), by Roberson and Noble (1968), Bullock et al. (1969), and Bullock (1972).

Thompson (1959) demonstrated that nuclear polyhedrosis viruses of the cabbage looper and the bollworm (*H. zea*) are not infective at rearing temperatures of 39° C or higher. Ignoffo (1966b) confirmed that temperatures above 55° C inhibit nuclear polyhedrosis virus infectivity in *Heliothis* spp.; but nuclear polyhedrosis virus of the yellowstriped armyworm, *Spodoptera ornithogalli* (Guénee), is infective at temperatures as high as 46° C. Similar results have been obtained for alfalfa caterpillar, *Colias eurytheme* Boisduval, infected by cytoplasmic polyhedrosis virus (Tanada and Chang 1968); for Indian meal moth infected by granulosis virus (Hunter and Hartsell 1971); for codling moth infected by granulosis virus (Pristavko et al. 1971); and for tobacco budworm infected by cytoplasmic polyhedrosis virus (Bullock 1972). Unfortunately for disease control, rearing insects at high temperatures can produce sterility and abnormal growth (Bullock 1972).

Obtaining healthy insects for rearing facilities

Obtaining healthy insects, when available, from other rearing facilities may be the simplest, most effective way to establish a disease-free culture (see, for example, Steinhaus 1953, Gast 1966, Brooks 1968, Helms and Raun 1971, and Tompkins and Cantwell 1975). When insects are brought in from the field, it should be assumed that

²Benzalkonium chloride.

³Methylbenzethonium chloride.

⁴0.25 g Mercuric chloride, 6.5 g sodium chloride, 1.25 ml hydrogen chloride, 250 ml 95% ethanol, and 750 ml sterile distilled water.

they may be contaminated, diseased, or parasitized. Using egg sources from areas free from obvious disease is desirable. But the past history of the insect population may not be known. Magnoler (1970b) collected gypsy moth egg masses from light infestations, which he presumed to be disease free, but disease might still have been present. Grisdale (1968) collected forest tent caterpillar egg masses in the fall, especially from areas of new infestation where the level of disease and parasitism was low; even so, eggs were often contaminated by polyhedrosis viruses.

Field-collected insects should be kept isolated or quarantined from the main colony or rearing stock until their state of health is ascertained. The incidence of disease can be determined by not sterilizing some of the field-collected material (Beckwith and Stelzer 1979). But generally, the field-collected insects (egg stage, preferably) should be disinfected to minimize or eliminate contaminants. Spatial isolation or quarantine may not be practical because of space limitations. In that case, other measures must be taken, such as rearing the insects individually or in small groups (Steinhaus 1953, Henneberry and Kishaba 1966). This method may be time consuming and cumbersome, but it will certainly minimize transmission of pathogens between insects.

Pasteur (1870) helped to save the silk industry from the ravages of pebrine, a protozoan disease caused by *N. bombycis*, because, like Osimo (in an 1859 publication cited by Steinhaus 1956), he recognized that the pathogen could be transmitted from one generation to the next within the egg. So he examined the adult females as a way of insuring healthy eggs; if microscopic examination showed spores in the moth, the moth and eggs were destroyed. If no spores were found in the moth's tissues, the eggs were saved and used for clean stock. This method is still used successfully among commercial breeders in Japan (Ishihara and Fujiwara 1965). And recently, variations of the Pasteur method have been successful in reducing or eliminating other protozoa (see, for example, Bucher and Harris 1961; Gast 1966; McLaughlin 1966, 1971; Jenkins et al. 1970; and Hamm et al. 1971) and viruses (see, for example, Henneberry and Kishaba 1966).

Diet treatments

Heat sterilization.—Little information exists on sterilization of insect diets. Ignoffo (1965) demonstrated that temperatures of 70°–75° C used in diet preparation reduce the total contaminant level. This reduction may not be enough, however, to eliminate the effects of dietary contaminants. At the Otis Methods Development Center, we boil the diet ingredients during processing; doing so increases the shelf life of the diet and reduces the level of

microbial contaminants (Bell et al. 1981) without hindering growth and development of the gypsy moth. I have found no differences in growth and virus yields between gypsy moth larvae fed this standard diet and those fed autoclaved diet (unpublished data). Similarly, Griffin et al. (1974) found no statistical difference between boll weevils reared on autoclaved (120°–125° C for 15–20 minutes) diet and those reared on diet that had been flash-sterilized (130°–144° C for 30 seconds). A flash-sterilization temperature of 151° C results in fewer and smaller weevils. At the U.S. Agricultural Research Service's Boll Weevil Research Laboratory at Mississippi State, Miss., where Griffin and his associates did this work, flash-sterilization was adopted because it requires less labor than autoclaving and saves time. Vanderzant (1975) demonstrated that growth of the tobacco budworm was not affected when its wheat germ diet was autoclaved; but she recommended flash sterilization because of its practicality.

Antimicrobial treatment.—Chemical preservatives, antimicrobials, are routinely added during diet preparation to insect diets susceptible to contamination and spoilage. These chemicals are the same as those accepted as food additives by regulatory agencies of the United States and most other Western countries (Chicester and Tanner 1968). Of these, the parabens, sorbates, benzoates, acetates, and propionates are most commonly used in insect diets (Singh 1977). Antibiotics are also used, especially the tetracyclines and streptomycin, for their bactericidal and bacteriostatic activities (Singh 1977).

Selection of antimicrobials will depend on the insect being reared and on the contaminant. Sodium benzoate is most active against yeasts and bacteria, and the parabens are most active against yeasts and molds. Sorbic acid and its salts have broad-spectrum activity against yeasts and molds. Several acetates (vinegar and purified acetic acid, sodium acetate, calcium acetate, potassium acetate, and sodium diacetate) are most effective against yeasts and bacteria (Chicester and Tanner 1968). Formalin is often used in diets to help control viruses (see, for example, Ignoffo and Garcia 1968, Vail et al. 1968, and David et al. 1969). In tests of several antimicrobials for control of bacteria and molds in gypsy moth artificial diet, sodium omadine was the most effective antibacterial compound, and sorbic acid was more effective than methylparaben, Formalin, or acetic acid. Methylparaben and sorbic acid were the most effective antifungal compounds; when used together, they prevented fungal growth (M. Shapiro, unpublished data).

Methylparaben is often used in combination with sorbic acid and/or formaldehyde for control of several microbes, particularly molds (see, for example, Gast and Davich 1966, Kishaba et al. 1968, and Howell 1971). Some re-

searchers (Nettles and Betz 1966, Burton and Perkins 1972) have reported these combinations to be ineffective at the concentrations used. Similarly, Shorey and Hale (1965) reported some resistance by molds to methylparaben and sorbic acid used alone. And Gifawesen et al. (1975), in assessing 37 fungicides for control of *A. niger* in a wheat germ and casein diet, found that one *A. niger* strain was resistant to methylparaben.

Seven of the fungicides studied by Gifawesen et al. (1975) were effective. And Ludemann et al. (1979), in testing other fungicides against resistant *Aspergillus*, found some effective up to 14 days.

Fumagillin has been found effective in controlling various protozoans: *Nosema apis* (Zander), by Katznelson and Jamieson (1952), Bailey (1953), Moffett et al. (1969), and Hartig and Przelecka (1971); *Nosema* spores, but not *M. grandis*, in boll weevils, by Gast (1966); and *P. pyraustae* in the European corn borer, by Lewis and Lynch (1969, 1970), Lewis et al. (1971), and Lynch and Lewis (1971). Fumagillin has also reduced incidence of *N. fumeranae* in spruce budworm larvae (Wilson 1972). And it has been used in combination with other antimicrobials to control *Nosema* spp. and other microbes (Hsiao and Hsiao 1973, Gilliam and Morton 1974). Another agent effective in suppressing *Nosema* spp. is benomyl⁶ (Shinholster 1974, Armstrong 1976).

Of the antibiotics, chlortetracycline is the one most often used. It controls yeasts and bacteria (Ignoffo 1963). The antibiotics that have been successful in screening tests against various micro-organisms include: streptomycin sulfate for boll weevil diet (Gast 1966); methenamine mandelate and nalidixic acid against *S. marcescens* (King et al. 1975); erythromycin against the bacterium *Leuconostoc mesenteroides* van Tiegham on boll weevil diet (Childress and Williams 1973); erythromycin thio-cyanate, erythromycin sulfate (Hitchcock 1964), and sugar solutions containing tylosin actage, tylosin tartrate, or sulfathiazole sodium (Hitchcock et al. 1970) against European foulbrood in honey bee colonies; Thipyrameth⁶ against the amoeba *Melameba locustae* King and Taylor in grasshoppers (Henry 1968); and maramycin against *Nosema apis* in honey bees (Moffett et al. 1969). I bioassayed *B. cereus*, *P. vulgaris*, *E. coli*, *E. aerogenes*, and *S. aureus* against streptomycin, tetracycline, and chlortetracycline on gypsy moth diet; all three antibiotics were active against the five bacterial species at concentrations of 0.05%–0.1%, except chlortetracycline, which was ineffective against *B. cereus*.

To be usable in insect diets, antimicrobials must work over a wide pH range and resist degradation during heating. The most effective pH range for sodium benzoate is 2.5–4.0; for the parabens, 3–9; for sorbic acid, up to 6.5; for propionic acid, 3–5; and for the acetates, up to 5.2 (Chicester and Tanner 1968). The parabens and sorbic acid are stable during autoclaving and flash sterilization (Hedin et al. 1974).

Insects have been, and are still being, reared on natural plant material. Often this plant material must be decontaminated. Afrikan (1960) reported soaking of mulberry leaves in bacteriostatic concentrations of streptomycin, Aureomycin (chlortetracycline), and tetracycline to reduce disease in the silkworm. Likewise, Zlotin (1965) suppressed disease in the gypsy moth by soaking acorns in 0.1% potassium permanganate.

Little use has been made of antimicrobial treatment to destroy or inhibit microbial contaminants already growing on the diet (spot treatment). At the U.S. Agricultural Research Service's Fruit Insects and Agricultural Engineering Research Unit at Yakima, Wash., Chawla et al. (1967) tested sodium hypochlorite, methylparaben, and sorbic acid both before codling moth eggs were implanted on the diet and after fungi developed. Only sorbic acid (1% solution) worked for the preimplantation treatment; but, once the fungi had developed, each antimicrobial controlled fungal growth on the diet. Sorbic acid was then used routinely in rearing the codling moth without harming the insects. The spot treatment must have been effective for the control of fungi, as it was still being used 4 years later (Howell 1971). Spot treatment was also used successfully in the rearing of the European pine shoot moth, *Rhyaciona buoliana* (Schiffmüller), at Yakima. Although this method is useful in some instances, it is more prudent to discard the contaminated containers.

Antimicrobials have biological activity against the insect as well as the microbe (Singh and Bucher 1971). Singh and House (1970) and Singh and Fowler (1973) defined a safe level of the antimicrobial agent as the concentration that does not make the insect take more than 25% longer than the control to grow and develop. The acceptable level will probably have to be determined for each insect. For example, Ouye (1962) recommended concentrations of 0.15% methylparaben and 0.05% Formalin in pink bollworm diet because higher concentrations increase duration of the larval and pupal stages. Moore et al. (1967) reported that use of methylparaben and potassium sorbate in boll weevil diet at concentrations greater than 0.5 ml inhibits egg hatch and larval growth. Henneberry and Kishaba (1966) reported that concentrations of methylparaben up to 8,000 p/m (parts per million) do not harm cabbage loopers, but sorbic acid concentrations greater than 4,000 p/m kill eggs. Other adverse effects on

⁶Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate.

⁶6% Sulfamethazine, 12% sulfathiazole sodium, and 8% sulfapyridine sodium.

cabbage looper growth and development include lack of silk formation in cocooning caused by butylparaben (*n*-butyl *p*-hydroxybenzoate) and increase of the larval period caused by high concentrations of sorbic acid, methylparaben, and butylparaben. Boush et al. (1968) reported death of progeny of black carpet beetle, *Attagenus megatoma* (Fabricius), and the beetle *Trogoderma parabile* Beal fed sorbic acid in the larval stage. Neilson (1973) reported that methylparaben, butylparaben, and sorbic acid hinder egg hatch of the apple maggot, *Rhagoletis pomonella* (Walsh). Gifawesen et al. (1975) reported thimerosal (merthiolate) as an effective mold inhibitor but toxic to tobacco budworm larvae; and Ludemann et al. (1979) reported that agricultural fungicides incorporated into the diet prolong larval development and decrease pupal weights. Ignoffo (1963a) reported that chlortetracycline (0.5 mg/ml) inhibits cabbage looper larval development, and, at 1.0 mg/ml, larvae fail to molt. Gast (1966) and Flint et al. (1972) found that boll weevils fed various levels of fumagillin develop abnormally. Fumagillin used to control *Nosema* also harms European corn borer (Lynch and Lewis 1971), alfalfa weevil (Hsiao and Hsiao 1973), and spruce budworm (Wilson 1974). Antimicrobial effects on insects may be immediate (toxic) or long term (ovicidal). These effects must be considered in any rearing system.

Conclusions

Steinhaus (1953) suggested the following principles that could be used to suppress or eradicate a disease and prevent its recurrence:

1. Whenever possible, diagnose the disease. Identify the contaminant or microbe.
2. If a disease is present in at least 25% of the insects, it is best to destroy the stock and decontaminate the facility and equipment. Reestablish the stock from a healthy culture if possible. In the case of chronic infections (for example, protozoan infections), selection of healthy insects may be feasible.
3. Cleanliness should be maintained constantly for the insect, the rearing environment, the equipment, and the food.

And Steinhaus (1968) observed that contaminant or pathogen suppression not only means sterilization and sanitation but also "new methods of prevention and therapeutics, more precise studies on the role of stressors (e.g., adverse temperature, humidity, food and space) in bringing about outbreaks of disease, and a greater understanding and application of what has been learned by those who for so long a time have been concerned about suppressing disease in the silkworm and the honey bee."

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Microbial Contamination in Insectaries

Occurrence, Prevention, and Control

By Peter P. Sikorowski¹

Introduction

Steinhaus and Martignoni (1970) defined contamination as the harboring of, or having contact with, micro-organisms without a relationship that is commensalistic, mutualistic, or parasitic. Microbial contaminants are usually composed of seemingly innocuous microbes. But animal pathology has shown that micro-organisms previously considered as innocuous, commensals, or contaminants may multiply extensively in the tissue of weakened hosts and cause disease that is often severe (Neter 1974).

About 450 species of phytophagous insects have been reared on synthetic diets (1977). Synthetic and semi-synthetic insect diets are usually complex media subject to spoilage by many species of bacteria and fungi (see, for example, Ignoffo 1966, Singh and House 1970a, Sikorowski 1975, McLaughlin and Sikorowski 1978, and others). Spoilage is the result of metabolic activity associated with microbial growth, causing catabolism of the media and release of products of digestion. The biochemical changes produced by microbes alter the nutritional value of the diets. Also, some bacteria and fungi produce toxins that may harm insects.

Humans are also affected by microbial contamination. Several airborne micro-organisms such as *Aspergillus*, *Pseudomonas*, and *Streptococcus* spp., which grow on almost any organic matter, are also human pathogens and present some hazard to employees in insect-rearing programs.

The cost of rearing insects can be greatly reduced by establishing an environmental sanitation program (Sikorowski 1975). Much information pertinent to laboratory rearing of insects has been obtained only recently, and most areas covered in this report merit further study. But research results to date suggest that sanitation will play an important part in mass rearing of most insects and that more attention will have to be given to enforcement of basic sanitary measures to assure volume production of healthy insects.

Occurrence of Microbial Contamination

Sources of contamination

Humans are the primary source of microbial contamination, and the level of contamination relates directly to activity and density of personnel (Favero et al. 1966, 1968; Runkle and Phillips 1969). The healthy human body harbors millions of micro-organisms on the skin in the mouth, respiratory tract, genitourinary tract and intes-

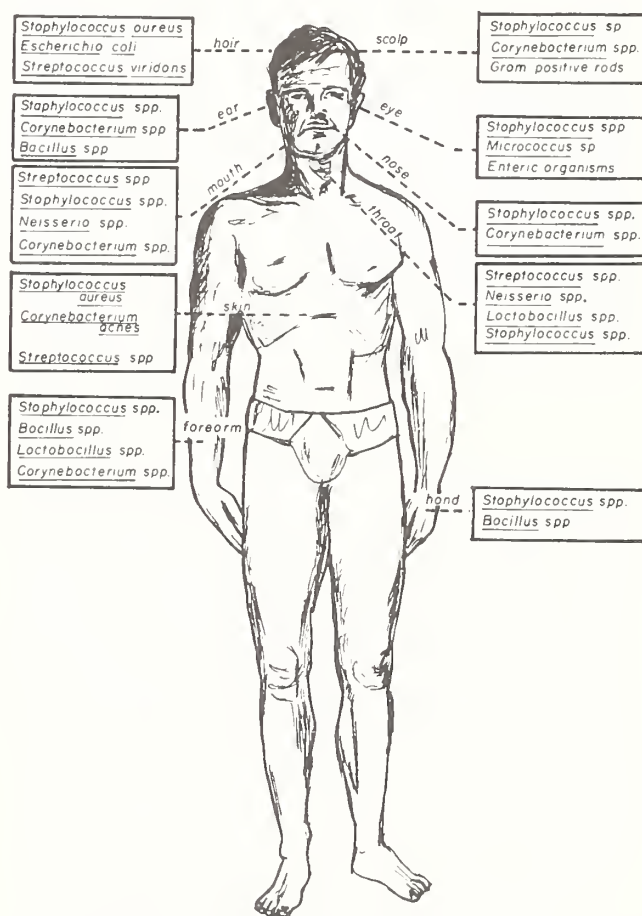


Figure 1.—Prevalent microbial flora of man (based on National Aeronautics and Space Administration 1969).

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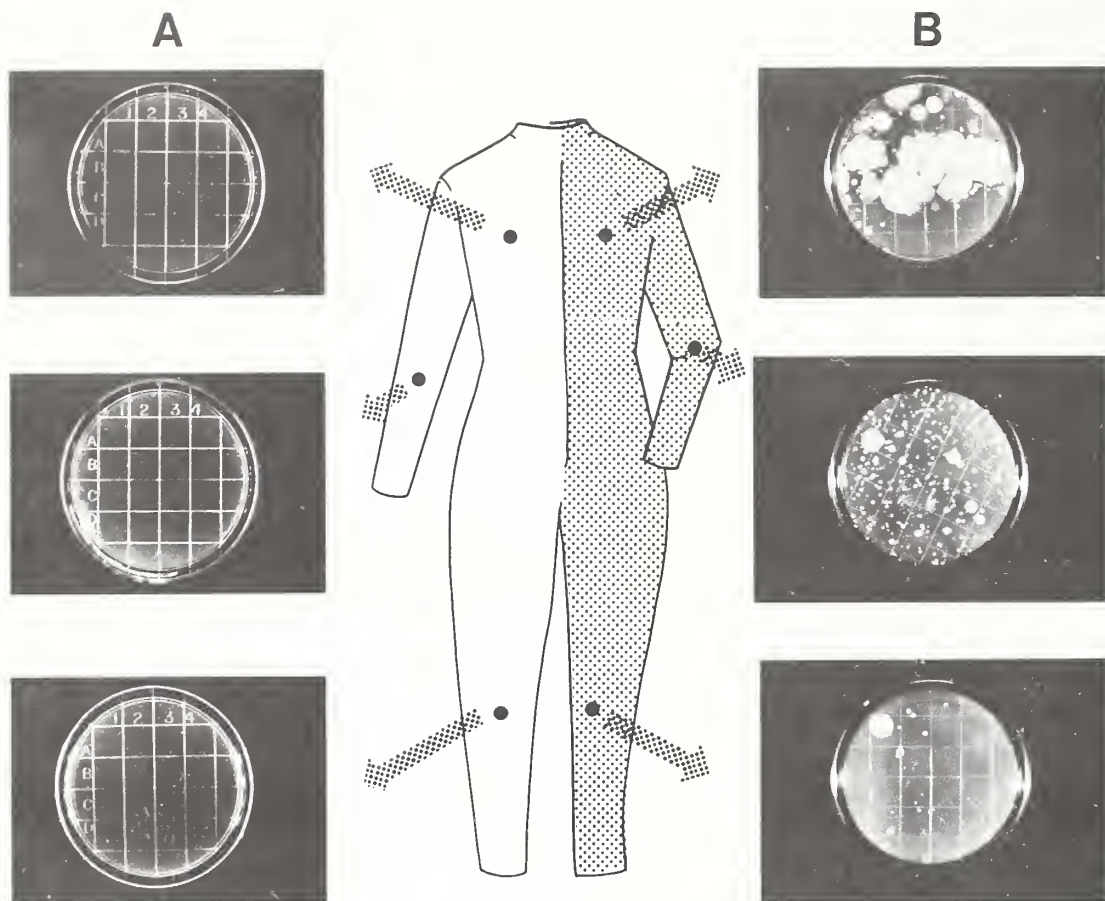


Figure 2.—Relative composition of microbes on (A) clean, freshly laundered uniform and (B) worn uniform.

times (fig. 1). Jawetz et al. (1978) arranged the normal microbial flora of the human body into two groups: The resident flora consist of fixed types of micro-organisms regularly found in a given area and in persons of a given age. The transient flora consist of nonpathogenic or potentially pathogenic micro-organisms that inhabit the skin and mucous membranes for hours, days, or weeks; it is derived from the environment, does not produce disease, and does not establish itself permanently on the surface. Jawetz et al. (1978) reported that constant exposure to and contact with the environment results in contamination of the skin with transient micro-organisms.

Smith and Bruch (1969) monitored healthy individuals who exercised naked for 30 minutes and found that each dispersed into the air 2-6 million viable micro-organisms. Sikorowski (1975) showed that freshly laundered and sterilized cotton uniforms do not provide an efficient bar-

rier for movement of bacteria through the cloth into the environment (fig. 2). The hair of 50 individuals (1 hair per person) had from several to many bacteria per hair. Cultures from hands almost always produced bacterial colonies even shortly after normal washing.

It is generally agreed that properly cared for equipment, instruments, walls, floors, etc. are minor sources of microbial contamination. The microbial content of the air in an area usually reflects the total microbial contamination of the surrounding area (Loughhead and Moffett 1971).

Normal flora of wild insects

Understanding the microflora of insects reared in insectaries is based on a knowledge of the normal flora of wild insects. The most commonly occurring, internally harbored micro-organisms in insects are bacteria or bacteria-

like forms that are found in Blattodea, Isoptera, Homoptera, Heteroptera, Phthiraptera, Coleoptera, Hymenoptera, and Diptera. Also, flagellates are found in wood-eating insects and yeast and yeast-like organisms in Homoptera and Coleoptera (Steinhaus 1949 and Chapman 1971). Steinhaus (1941) studied the bacterial flora of 30 species of insects and isolated 83 strains of bacteria, 2 strains of yeasts, and 2 molds from the insects. And, in most cases, the species of bacteria found in the several specimens of any given insect species were surprisingly constant. A study of the internal microbial flora of 2,016 unfed specimens of the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles, showed that only 1.6% of the adults harbored bacteria (Steinhaus 1942). Over 75% of field-collected adult boll weevils, *Anthonomus grandis grandis* Boheman, of various ages examined by Sikorowski et al. (1977) and McLaughlin and Sikorowski (1978) had 100 or fewer bacteria per insect. Antibacterial constituents (such as gossypol, caryophyllene, gallic acid, and tannins) found in host cotton plants are responsible for the low bacterial content of wild weevils (Hedin et al. 1978). Greenberg (1962) reported that 73% of house flies, *Musca domestica* Linnaeus, and 54% of stable flies, *Stomoxys calcitrans* (Linnaeus), are germ free at emergence from the puparium. He concluded that sterilization of the digestive tract is the usual consequence of metamorphosis.

Insects that feed on diets deficient in certain elements usually have micro-organisms associated with them. This suggests that the micro-organisms make good the diet deficiencies. But, in many cases, the precise nature of the micro-organisms is not known (Chapman 1971; for reviews of the literature about micro-organisms of healthy insects, see Steinhaus 1940 and Brooks 1963).

Bacterial flora of insectary-reared insects

Insects reared in an insectary are often contaminated with different species of aerobic and anaerobic bacteria (see, for example, Ignoffo 1966; Sikorowski 1975; Davis 1976; McLaughlin and Sikorowski 1978; Smalley and Ourth 1979; Bell et al. 1981; and others); relative composition of contaminants often changes from day to day. I have isolated, from apparently normal insectary-reared boll weevils, many taxonomically unrelated species: from the family Micrococcaceae, *Micrococcus luteus* (Schroeter) Cohn and *Staphylococcus aureus* Rosenbach; from Streptococcaceae, a *Streptococcus* sp. and *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem; from Bacillaceae, *Bacillus sphaericus* Meyer and Neide; from Lactobacillaceae, *Lactobacillus plantarum* (Orla-Jensen) Bergey et al.; from Pseudomonadaceae, *Pseudomonas aeruginosa* (Schroeter) Migula; from Enterobacteriaceae, *Enterobacter aerogenes* Hormaeche and Edwards; and

from the Coryneform group, *Corynebacterium humiferum* Seliskar (unpublished data). *Pseudomonas aeruginosa*, *M. luteus*, a *Streptococcus* sp., *Serratia marcescens* Bizio, *S. rubidaea* (Stapp) Ewing et al., *Aspergillus niger* van Tieghem, *A. flavus* Link, *Rhizopus nigricans* Ehrenberg, a *Cladosporium* sp., a *Fusarium* sp., and two yeast species were isolated from laboratory-reared larvae of *Heliothis* spp. by Bell et al. (1981). McLaughlin and Sikorowski (1978) tested 35 bacterial cultures, mostly from the American Type Culture Collection, for their ability to grow on the boll weevil diet. Five of 15 human pathogens and 7 of 20 saprophytes developed on the diet.

Effects of bacterial contamination on insects

The number of insects that emerge from contaminated diets is influenced by species of micro-organisms present, stage of the insect at the time of contamination, temperature, pH of the medium, and perhaps by other factors. For example, in one study, I found that boll weevil diet contaminated with *S. aureus* at the time of egg implanting produced 57% as many weevils per petri dish as did uncontaminated diet, 57% for diet contaminated 3 days after larval hatch, and 71% for diet contaminated 6 days after larval hatch (unpublished data). Likewise, contamination of the diet at the same time intervals with a *Streptococcus* sp. provided only 76%, 64%, and 80% as many weevils per petri dish as did uncontaminated diet. The normal developmental time of 13 days from egg to adult weevil extended to 14 or 15 days on diets contaminated with some species of bacteria. Fast-growing bacteria such as *Leuconostoc* spp. may overgrow an area of diet of about 700 mm² in 24 hours and smother all the larvae on the dish. The staphylococci (enterotoxin producers) may harm larvae.

Maeda et al. (1953) reported that, under aseptic conditions, larvae of the oriental fruit fly, *Dacus dorsalis* Hendel, develop equally well in diets of pH 4.5, 5.5, 7.0, and 8.0. But, when aseptic techniques are not followed, any medium with a pH higher than 5.5 has poor larval growth because bacterial contamination is heavy. At room temperature, the pH of diet for phytophagous insects decisively influences the microbe's ability to flourish on it. For most bacteria, a minimum pH is 4.5–5.0, maximum 8.0–8.5, and optimum 6.5–7.5 (Oginsky and Umbreit 1955). Approximate minimum and maximum pH values for molds are 1.5–11.0, and for yeasts 1.5–8.5 (Jay 1978). But the metabolic activity of a bacterium may produce several acidic and basic products from the components of the medium. The release of such substances during bacterial growth would shift the pH of the medium. In general, insect media with pH 4 and above are subject to bacterial spoilage. Media with pH 4 and below may undergo mold and yeast spoilage.

G. A. Virginio (personal communication) found that infection of lepidopterous larvae with microbial contaminants causes high mortality and delay in larval development. He also observed that, when infections do not kill the larvae, pupae and adults are abnormally small.

McLaughlin and Sikorowski (1978), Sikorowski and Thompson (1979), and MacGown and Sikorowski (1980) showed destructive effects of bacterial contamination on the brush border of the midgut epithelium of the boll weevil. In contaminated weevils, the brush border is frequently ulcerated and overgrown by bacteria. In testing how bacteria affect pheromone production in the boll weevil, Gueldner et al. (1977) and G. Wiygul and P. P. Sikorowski (unpublished data) measured the amount of pheromone of the male boll weevil in the feces; and P. A. Hedin and P. P. Sikorowski (unpublished data) measured the amount in body homogenate. More pheromone was always isolated from the frass and body homogenate of uncontaminated weevils than from contaminated weevils. Pheromone production, and therefore attractiveness of the sexually sterile weevil, is a desirable characteristic in mass-reared weevils; the quality of weevils that are bacterially contaminated is reduced to significantly below normal, and these weevils would be expected to have a greatly diminished competitive value. Thompson and Sikorowski (1978) and Thompson et al. (1977) studied how a *Streptococcus* sp., *Micrococcus varians* Migula, and *E. aerogenes* contamination affect amino and fatty acid contents of boll weevils. They reported that, except for tyrosine and glutamine in the females, all amino acids analyzed are found in greatest amounts in weevils free of bacterial contamination. The average reduction of amino acids due to bacterial contamination is 33% in males and 52% in females. The high level of tyrosine and glutamine in contaminated female weevils was not explained. Comparison of individual fatty acids between the groups shows a decrease of up to 76% in highly contaminated insects ($\geq 210,000$ bacteria per insect). Hurej et al. (in press) reported that bacterially contaminated boll weevils react differently to insecticide treatment than do those free of contamination. The bacteria tested decreased mortality in all groups of boll weevils treated with methyl parathion.² The effects of contamination on the toxicity of mirex³ varied with the species of bacteria. So bacterially contaminated boll weevils have differed from uncontaminated insects in all variables tested. This, to my knowledge, is the first attempt to evaluate the effects of bacterial contamination on the quality of artificially reared weevils or any other insectary-reared insect.

Prevention

To a rearing program, contamination can mean increased expense, poor quality of insects, high mortality of all stages, additional workload, and loss of confidence in the work. To prevent contamination, insect diets must be of good sanitary quality, so they must be free of hazardous micro-organisms, and saprophytes should be at acceptable levels. To overcome the problems caused by contamination and to prevent its recurrence, insectary personnel must be trained in the need for sanitation, and regular sanitary measures must be established and maintained. Monitoring techniques are used to demonstrate that the system can reliably keep microbial contamination below undesirable levels.

Training of insectary personnel

Personnel must be trained to understand rearing operations and that their own potential for contaminating the area is high. Training programs for insectary workers should begin with a discussion of the quality of insect required. Most people without basic training or experience in sanitary microbiology have difficulty comprehending the presence of microbes on apparently clean hands, garments, and equipment. Sikorowski (1975) described inservice training conducted in the U.S. Animal and Plant Health Inspection Service's Robert T. Gast Boll Weevil Rearing Facility at Mississippi State, Miss., to familiarize all employees with the basic concepts of sanitary biology. A major part of the training was the conducting of experiments that showed the presence of microbes on unsanitized floors, clothing, shoes, and hair, and in the breath.

Sanitary measures

The objective of establishing an environmental sanitation program is to eliminate contamination that interferes with the rearing of healthy insects. The level of cleanliness required must be decided on before the needed techniques are determined. Many researchers have described different sanitation programs used in their insectaries (see, for example, Steinhaus 1953; Sikorowski 1975; Davis 1976; Martignoni and Iwai 1977; and Bell et al. 1981). Most of these include in their sanitary measures personal hygiene; maintenance of a clean, sanitary environment by proper housekeeping; and various methods of sterilizing and sanitizing insectary equipment and environment.

Personal hygiene.—Micro-organisms are usually shed with the human skin scales they are attached to. Taking a shower degerms the skin by causing about a five-fold increase in the rate of shedding micro-organisms. The high population of normal flora returns in 1–2 hours after thorough showering (National Aeronautics and Space Ad-

²O,O-Dimethyl O-(p-nitrophenyl) phosphorothioate.

³Dodecachlorooctahydro-1,3,4-metheno-1H-cyclobuta[cd]pentalene.

ministration 1969). The major purpose of clean uniforms is to limit the passage of skin microbes to the environment; some microbes escape when the uniform is not properly closed. Frobisher et al. (1969) reported that the human mouth and throat constantly contain many kinds of micro-organisms. The micro-organisms multiply in the secretions of the nose and oral cavity and between the teeth. Commonly found are streptococci, staphylococci, pneumococci, and spirochetes. Sikorowski (1975) reported that personnel concerned with handling insect diets and adult boll weevils and with planting eggs were required to wear caps and wash their hands frequently with Surgi-Bac (Economics Laboratory, St. Paul, Minn.). Workers handling weevil diets were also required to wear hair caps and face masks.

Housekeeping.—Litsky and Litsky (1968) and many other workers in the field of microbial contamination have demonstrated that flooding and wet-vacuuming is the most effective method for cleaning and disinfecting floors. Portable vacuum cleaners may be used only if the vacuum cleaner exhaust is filtered to the same extent that the air is filtered through the air-filtration system in the room. Sweeping and dry-mopping might stir up micro-organisms and should be avoided (Nagasawa et al. 1970). Runkle and Phillips (1969) recommend elimination of sweeping through the use of dry or wet pickup vacuum cleaners with high-efficiency exhaust air filters. The general housekeeping routines are given by Sikorowski (1975), Davis (1976), and Martignoni and Iwai (1977).

Sterilization of equipment.—"Sterilization" means the destruction of all life. Heat is the most reliable and universally applicable method of sterilization and should be the method of choice whenever possible. All living organisms can be rapidly destroyed by steam under pressure. The greatest problems for steam sterilization are moisture penetration, air removal and entrapment, heat and moisture damage, and thorough wetting (Ernst 1977). (See Martignoni 1978 for the principles and proper use of the autoclave and Hedin et al. 1974 for how autoclaving and flash sterilizing affect potassium sorbate and methyl *p*-hydroxybenzoate (methylparaben) in boll weevil diets.)

Ultraviolet light.—The most effective antibacterial wavelength of ultraviolet light is between 240 nm and 280 nm; the optimum is about 260 nm. Ultraviolet light kills bacteria because it is absorbed by and damages the DNA (Willett 1976). Ultraviolet light has poor penetrability; it does not penetrate solids, and it penetrates liquids very slightly. Micro-organisms are protected by dust in the air and by dust on the ultraviolet-light bulbs; and clumping of microbial cells (microbial cells attached to dust) allows those in the central part of the clump to escape the effect of ultraviolet light. At the R. T. Gast Rearing Facility,

ultraviolet light failed to produce uniform results in the control of airborne micro-organisms in ultra-violet light passthrough box units used for passage of materials from one area of the laboratory to another.

Sanitizing equipment.—Large equipment, such as a diet-processing apparatus that is permanently attached to the walls, should be sanitized immediately before and just after use to prevent entry of microbes into the apparatus. Small instruments that cannot be autoclaved may be sanitized by immersion in sanitizing solution. Procedures used to sanitize equipment are given by Sikorowski (1975), Martignoni and Iwai (1977), and Griffin et al. (1979).

Air disinfection and air filtration.—Air filtration is one of the most important tools against microbial contaminations. Many different types of air-cleaning equipment are available—gravitational, inertial, filtration, washing, and electrostatic precipitation. Filtration is usually recommended when maximum removal of bacterial particles from the air is required.

Particles carrying bacteria are usually greater than 4 μ m in diameter, with a median of 10–20 μ m, although single spores of bacteria are often not associated with dust carriers (National Aeronautics and Space Administration 1968). HEPA (high-efficiency particulate air) filters remove 99.97% of particles that are 0.3 μ m in diameter and larger. This laminar airflow prevents microbial accumulation in a room atmosphere. Laminar-airflow devices can be classified as follows: horizontal-airflow units, vertical-airflow units, and curvilinear-airflow (change in the direction of the air during its flow) units. In the R. T. Gast Rearing Facility, air in the egg-implanting area has been filtered with considerable success through HEPA filters having a 0.3- μ m pore size (Sikorowski 1975). After several years' experience in the use of laminated airflow, I find that this method is one of the most useful tools in controlling microbial contaminants. (See Phillips and Runkle 1973 for a general discussion of principles of laminar airflow and its possible biomedical applications.)

Fogging a room.—Greene (1970), in discussing the advantages and disadvantages of fogging a room with germicides, reported that heavy airborne particles settle by gravity while the room is left undisturbed; so the objective changes from air decontamination to surface decontamination. He also found that the lighter microbial particles have only a remote chance of contacting a germicidal droplet and of staying with it in a moist state long enough to be killed. Friedman et al. (1968) demonstrated that spray-fogging hospital rooms with a quaternary ammonium disinfectant effectively reduces the number of detectable airborne and surface bacteria.

They also observed that fogging with quaternary ammonium disinfectant does not produce local or generalized irritation in housekeeping personnel and technicians. F. M. Davis' (unpublished data) and Sikorowski's (1975) studies also show that spray-fog disinfection with quaternary disinfectant usefully decreases airborne micro-organisms.

Antimicrobial compounds.—Below is a list of the major groups of antimicrobial compounds used for cleaning and sanitizing.

1. Iodophors.—Iodophors are combinations of iodine and a carrier. Their activity is based on the slow release of iodine. Iodine is effective against a wide variety of micro-organisms; it is lethal to viruses, bacteria (including spore formers), fungi, protozoa, and algae. But recent works suggest that iodine is less sporicidal than hypochlorite (Trueman 1971). Mikrokylene (Economics Laboratory, St. Paul, Minn.) is an iodophor detergent-disinfectant claimed by the manufacturer to be effective against the spores of *Bacillus subtilis* (Ehrenberg) Cohn and *Clostridium sporogenes* (Heller) Bergey et al. at a concentration of 2.0% with a temperature of 80° C and an exposure time of 30 minutes.
2. Quaternary ammonium compounds.—The bactericidal power of the quaternaries is high against Gram-positive bacteria, but they are less effective against Gram-negative bacteria. They are not sporicidal. They also have bacteriostatic characteristics far beyond their bactericidal concentration. They are also fungicidal and can destroy certain sanitizing agents. Other features are low mammalian toxicity, low corrosiveness, and combined properties of germicidal and detergent activity. They are stable, odorless, do not stain, dissolve easily in water, and are inexpensive. Commercial quaternary ammonium compounds include Bradophen⁴ (Ciba-Geigy Ltd., Basel, Switzerland), Mikro-quat⁶ (Economics Laboratory, St. Paul, Minn.), and Roccal⁶ and Quatsyl 256⁷ (National Laboratories, Montvale, N.J.). (See Lawrence 1950 and Petrocci 1977

for reviews of quaternary ammonium compounds and Martignoni and Milstead 1960 for discussion of their use in surface sterilization of insects.)

3. Phenolic compounds.—Phenol in high concentrations is a protoplasmic poison that penetrates and disrupts the cell wall and precipitates the cell proteins. At lower concentrations, it inactivates the essential enzyme systems. Phenolic compounds are effective against vegetative cells of bacteria, and, at high temperatures, against fungal and bacterial spores. Commercial phenolic compounds include Amphyl⁸ (National Laboratories, Montvale, N.J.) and Con-O-Syl⁹ (Johnson and Son, Racine, Wis.).
4. Sodium hypochlorite.—Hypochlorites (calcium and sodium) are the oldest and most widely used chlorine compounds in the field of chemical disinfection (Dychdala 1977). They are effective against a wide spectrum of micro-organisms, including spore formers. The sodium hypochlorite solutions range in concentrations from 1% to 15% with 1%–5% available chlorine products used domestically and stronger solutions industrially. Today, hypochlorites are used for microbial control in households, hospitals, schools, restaurants, food-processing plants, dairies, canneries, wineries, and beverage bottling plants (Dychdala 1977). Hypochlorites are also extensively used as disinfectants in rearing facilities. Sodium hypochlorite solution is available in most grocery stores as a laundry bleach (for example, Clorox and Purex).
5. Formalin.—Formalin is commonly a 37%–40% solution of formaldehyde in water. This antimicrobial compound is sporicidal, bactericidal, and, at lower concentrations, bacteriostatic. Formalin contains certain stabilizers, 8%–15% methanol being the one most commonly used, to prevent the formation of solid polymers if the solution is chilled or is kept for a long time (Phillips 1977). Formalin is most effective at high relative humidity. It penetrates very slowly; therefore, its application should be limited to surface sterilization. Although Formalin has been widely used for at least 80 years, it should be used only when no other method is available. (For more information about Formalin, see Hoffman 1971 and Trujillo and David 1972.) Recently, a New York University study has provided

⁴Benzyl-dodecyl-bis-(2-hydroxyethyl)-ammonium chloride.

⁵Active ingredients: alkyl (50% C₁₄, 40% C₁₂, 10% C₁₆) dimethyl benzyl ammonium chlorides, 9.0%; trisodium ethylenediamine-tetraacetate, 0.4%.

⁶Benzalkonium chloride.

⁷Active ingredients: octyl decyl dimethyl ammonium chloride, 3.750%; dioctyl dimethyl ammonium chloride, 1.875%; didecyl dimethyl ammonium chlorides, 1.875%; alkyl (50% C₁₄, 40% C₁₂, 10% C₁₆) benzyl dimethyl ammonium chloride, 5.000%; tetrasodium ethylenediamine tetraacetate, 3.420%; isopropyl alcohol, 3.000%; ethyl alcohol, 1.000%.

⁸Active ingredients: *o*-phenylphenol, 10.5%; *o*-benzyl-*p*-chlorophenol, 5.0%.

⁹Active ingredients: *o*-phenylphenol, 11.75%; *o*-benzyl-*p*-chlorophenol, 7.82%; sodium dodecylbenzenesulfonate, 3.17%; tetrasodium ethylene diamine tetraacetate, 2.96%; isopropyl alcohol, 2.60%; essential oils, 0.30%.

decisive confirmation of industry findings that formaldehyde is an animal carcinogen (Sun 1981).

6. **Soaps.**—Many different soaps have bacteriostatic or bactericidal properties. The germicidal soaps are ordinary toilet soaps with various antimicrobial agents added to a maximum concentration of 2%. Phenol derivatives, especially creosols and bis-phenols are often mixed with soaps. (For more information on soaps, see Frobisher et al. 1969 and Hamilton 1971.)

Contamination-monitoring program

Contamination control manages microbial contamination at desired levels. The monitoring program evaluates the effectiveness of the contamination-control program. One of the earliest systems for monitoring microbial contamination is the program, described in Sikorowski (1975), that was used for several years in the R. T. Gast Boll Weevil Rearing Facility. It included microbiological sampling of air; larval diet; weevil eggs; equipment, walls, floors, and surfaces; and adult weevils. What follows is a description of this program.

Air microbial content.—Most of the important bacteria in the food industry range from 2 to 4 μ m long and 0.5 to 1.5 μ m in diameter. So 100 liters of air were drawn through a 0.45- μ m membrane filter supported in a filter assembly. The filter was then placed in a 12- by 54-mm plastic petri dish with an absorbent pad saturated with mycological or plate-count broth and incubated at 35° C for 24 hours. Afterward, the filters were removed from the dishes, air-dried, stained with methylene blue solution (0.5% methylene blue in 100% ethyl alcohol) for 30 seconds and redried. A 1- by 2-cm strip was cut out from the central portion of the filter, placed on the slide, and examined with a microscope at \times 100. (Runkle and Phillips 1969, Dark and Harper 1972, and others have described techniques used in monitoring air microbial content.)

Sterility of larval diet.—Twenty-ml samples of diet were collected aseptically in sterile petri dishes every 15 minutes during the operation time. The dishes were examined after 24–48 hours incubation at 37° C.

Sterility of eggs.—From every 2,000-ml bottle of eggs (150 ml of eggs + 1,750-ml of egg-implanting solution), 5 samples of 2,000 eggs were implanted in each petri dish with sterile trypticase soy agar, then incubated for 72 hours at 37° C. A Gas Pak Anaerobic System (Baltimore Biological Laboratory, Cockeysville, Md.) was used for isolation of anaerobic bacteria. A dish with one or more colonies indicated egg contamination.

Micro-organisms on surfaces.—Two methods were used for detection of bacteria on surfaces: the swab-rinse method and the agar-contact method. The swab-rinse method was originally developed by Manheimer and Ysanez in 1917. It has had many modifications and is now widely used. A cotton-tipped sterile swab is rubbed over the surface of the object to be sampled. The tip of the swab is broken into a tube containing sterile dilutant, the tube is shaken, and the rinse fluid is plated on an appropriate culture medium. Care is taken to avoid contaminating the swab. Patterson (1971) summarized the major disadvantages of this method: there is often poor recovery of bacteria from the surfaces sampled, results are not reproducible between different workers or laboratories, and cotton retains micro-organisms.

The agar-contact method uses a Rodac plate, a modified petri dish that contains a raised nutrient-agar bed. The plate is placed in contact with the tested surface, and the samples are usually incubated for 48 hours at 37° C before the colonies are counted. This method monitors surface cleanliness. Trypticase soy agar with lecithin and polysorbate 80 medium is prepared for detection of surface contamination. Lecithin and polysorbate 80 inactivate residual disinfectant collected with the specimen.

The microbial contamination in the R. T. Gast Rearing Facility varied with the area tested. In the egg-implanting and larvae holding rooms, where air was filtered through the HEPA filters, the bacterial count was less than 10 colonies per plate. The tests were made immediately after the floor had dried following wet cleaning and before traffic was permitted.

Adult examination.—The criteria used by Sikorowski (1975) to evaluate quality of adult boll weevils were amounts of bacteria, protozoa, viruses, and yeast.

1. **Bacteria.**—A sample of 20 adult weevils was frozen (–20° C) for 15–20 minutes, surface-sterilized with 0.5% sodium hypochlorite solution for 5 minutes, and washed in 2 changes of sterile water (5 minutes each); each weevil was blended in 40 ml of sterile water for 30 seconds. Forty ml of the homogenate was mixed with 30 ml of melted agar (45° C) and poured into petri dishes. After incubation, the colonies were counted.
2. **Protozoa (Microsporidia).**—A sample of 25 adult weevils was blended in 25 ml of water. The suspension was then filtered through cheesecloth and centrifuged at 3,000 revolutions per minute for 10 minutes. The resulting pellet was resuspended in a small quantity of water, smeared on slides, and examined under a phase-contrast microscope. (For more information about

diagnostic techniques used in identification of insect-pathogenic protozoa see Hazard 1975 and Poinar and Thomas 1978.)

3. Viruses.—If a virus disease was suspected, the host tissue was examined with a light or electron microscope as required. Insect viruses can be divided into two broad groups: occluded viruses (nuclear and cytoplasmic polyhedroses, the granuloses, and the insect pox diseases); and free viruses, which are not embedded in a crystalline matrix. Inclusion bodies of the occluded viruses can be easily detected with light or phase-contrast microscopes. For detection of nonoccluded viruses, an electron microscope must be used. M. E. Martignoni (personal communication) has suggested that an infectivity test would be a more general and probably cheaper method for detection of nonoccluded viruses.
4. Yeasts.—If a yeast contamination was suspected, the methods given by Poinar and Thomas (1978) were used.

Control of Microbial Contamination

Use of antimicrobials

Singh and House (1970b) reported that, to prevent microbial contamination in synthetic diets of 50 different phytophagous insects, chlortetracycline has been used in 19 diets, formaldehyde in 18, methyl *p*-hydroxybenzoate in 58, sodium benzoate in 6, and sorbic acid and its salts in 42. Singh and House (1970a, 1970b, 1970c) and Singh and Bucher (1971) studied how 21 antimicrobials mixed with synthetic diets (meridic, or chemically defined) affected development of *Agria housei* Shewell. Singh and House (1970b) concluded from their work that the safe level for each antimicrobial compound and insect should be determined for each diet-insect combination. To minimize decreases in insect quality, the smallest effective amounts of an antimicrobial compound should be used in the diet. Bell et al. (1981) incorporated Aureomycin (chlortetracycline), benomyl,¹⁰ sorbic acid, and methyl *p*-hydroxybenzoate, in various combinations, into lepidopterous larval diets to inhibit bacteria and fungi. They concluded that benomyl and sorbic acid combinations provide the best protection against microbial contaminants in the specific diet tested. Bell et al. (1959) studied how sorbic acid affects 66 molds, 32 yeasts, and 6 species of lactic acid bacteria and found that all grow in the presence of 0.1% sorbic acid at pH 7.0. The molds and yeasts are inhibited when pH is lowered to 4.5, but not all bacteria are inhibited until it is lowered to 3.5.

Greany et al. (1977) reported that high pupal mortality experienced during laboratory rearing of *Opius longicaudatus* (Ashmead), a parasitoid of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), was mainly caused by opportunistic pathogens, *S. marcescens* and *P. aeruginosa*. Their study showed that stress caused by parasitism and high rearing temperatures contributed to deaths caused by bacteria. They also presented evidence that methenamine mandelate chemotherapy had no prophylactic effect; instead, potentially harmful side effects (aberrant premating sounds from the fly), were caused by incorporation of this antibiotic in *A. suspensa* larval medium. Control was accomplished by upgrading the cultural conditions rather than by the use of antibiotics.

Chawla et al. (1967) described a surface treatment with antimicrobials to control fungi on wheat germ diets used for mass rearing the European pine shoot moth, *Rhyacionia buoliana* (Schifferrmüller), and the codling moth, *Carpocapsa pomonella* (Linnaeus). Sikorowski et al. (1980) described a method that called for mixing antimicrobials with sand or corncob grit and depositing the mixture on the surface of the growth medium to shield boll weevil larvae from microbial contamination. The results of the study showed that: antibiotics can be applied to the medium to shield the larvae from microbial contamination without detectably hindering boll weevil development, egg production, hatch, and pheromone production; and the standard diet overlain with medicated sand yields 86%–97% newly emerged weevils with less than 500 bacteria per weevil, while diet with unmedicated sterile sand yields only 18%. Sikorowski et al. (1980) believe that antibiotics should be used only under certain unusual circumstances when sanitary measures are temporarily interrupted. Prolonged use of antibiotics may lead to the selection of resistant strains of bacteria.

Upgrading existing sanitary measures

When enforcement of sanitation programs was relaxed in the R. T. Gast Rearing Facility, the cleanup procedure deteriorated because personnel overlooked the basic sanitary measures (Sikorowski 1975). Clearly, then, in selection of insectary workers, one should be assured of the ability of the individual to perform the operation. The worker must also be able to do this work so that the essential cleanliness is not compromised. The worker must know contamination-control principles and be aware of the consequences of each action or inaction.

The furniture and equipment selected for the insectary should be chosen to minimize maintenance and cleaning. A large selection of cleanroom furniture, used in food industries, hospitals, etc., is being marketed today. Work surfaces should be noncorrosive and resistant to heat,

¹⁰Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate.

moisture, and abrasion. Stainless steel, because of its durability and resistance to most corrosive cleaning and sanitizing agents, is most frequently used for surfaces that contact diet. Consideration of sanitary requirements in the design of the equipment results in easier cleaning and sanitizing, thereby saving labor and materials.

Conclusions

Microbial contamination could be described as the occurrence and persistence of microbes in an environment where they are not wanted. Synthetic and semisynthetic insect diets may provide growth media for an almost infinite number of microbe species. The microbial contaminants may have diversified effects on insects, depending on vigor, age, composition of medium, etc. For example, a change in the host resistance to microbial infection, caused by stress, may raise a nonvirulent bacterial contaminant to the level of a disease-producing microbe. Among the more common problems associated with microbial contamination are: high mortality of young instars, prolonged developmental time, diminutive pupae and adults, reduced pheromone production, reduced amino- and fatty-acid synthesis, wide fluctuations in the quality of insects, and direct pathological effects. An environmental sanitation program, then, should eliminate contamination that interferes with the rearing of healthy insects. I see a trend toward more involvement of insect pathologists in the field of insect rearing. The control of microbial contaminants in insectaries often presents problems for which the field of insect pathology offers solutions.

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Section 5

Production, Use, and Quality Testing in Insect Rearing

The intent of this section is to convey the state of the science of insect rearing. The papers describe representative rearing systems for production of insects to be used for research, development, and biological control. We become aware of how complex it is to integrate the modern technologies of genetics, engineering, microbiology, and dietetics and to coordinate the principles of quality control and management with basic insect biology. We benefit from scientists who have many years of experience in rearing insects and who have firsthand knowledge of the advances made in the systems they describe. Each paper sets the priorities for maintaining the integrity of each rearing program. And some authors suggest ways to improve rearing capability. The following recommendations summarize the major problems and challenges addressed by the authors of this section.

The control of bacteria and fungi, like a persistent hypha, is common to every rearing system described in this section. Efforts to prevent, mitigate, eliminate, and manage microbial contaminants require much of the resources available to any rearing program. Contamination can eradicate an insect colony and years of work. And, even in less extreme cases, the subtle effects of sublethal microbial infections on the development, behavior, and reproduction of an insect may significantly impair its use for any purpose. Efficient methods are needed to sample for, and determine the effects of, sublethal infections. And, just as important, management guidelines for preventing contamination in the first place must be developed and documented.

Insects reared in the laboratory can differ significantly in quality from those in the wild parent population. But little is known about population dynamics and behavioral responses of most laboratory-reared species; indeed, there are relatively few published tests for deriving such information. Results of studies that use laboratory-reared insects without comparing their performance with their wild counterparts must be interpreted narrowly. Because

various laboratory cultures are used worldwide in many ways, standardized tests must be developed. And studies must be done to provide reference descriptions of how established insect colonies that have been reared with standard techniques respond to those techniques. The availability of uniform testing procedures and performance standards would promote communication and cooperation among scientists working with the same species, increase our understanding of the biology and behavior of the species, and enhance our ability to monitor and manage flaws in our rearing systems that lower the quality of insects produced.

The development of artificial diets has pioneered advancements in insect colonization. The renewed emphasis on biological control of agricultural pests insures that dietetics will continue to be important in the development and evolution of standardized rearing systems. There are several crucial areas in dietetics that need support and investigation. First, the quality of diet ingredients must be more closely defined, and tests to monitor ingredient quality should be available to the insectary manager. Second, the effects of various preparation procedures on diet quality and stability must be described and documented. Finally, large-scale rearing and use of many potentially effective entomophagous arthropods is prohibited by cost and difficulty of rearing both host and parasite. A concentrated effort is needed to develop artificial diets for entomophages; investigation of *in vitro* rearing systems would be especially appropriate.

The advances in genetic manipulation, in mechanization of rearing procedures, and in insectary design and management testify to the creative ingenuity of scientists to design and implement complex, cost-effective rearing systems that safeguard insect quality and quantity and the health and safety of insectary personnel. Developing such systems is the challenge for those people involved in production, use, and quality testing of laboratory-reared insects.

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Production of the Gypsy Moth, *Lymantria dispar*, for Research and Biological Control

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Introduction

The first attempt to rear the gypsy moth, *Lymantria dispar* (Linnaeus), in the laboratory in the United States occurred in 1868 and resulted in its escape and establishment in Medford, Mass. This notorious European forest pest was brought to the United States from France by Professor Leopold Trouvelot, an astronomer and naturalist who was trying to breed a new silkworm (Medford Mercury Press 1906). His experiment led to the creation of a pest-control industry that has flourished since the first major outbreak in 1889.

After escaping from a window of Trouvelot's basement laboratory, the polyphagous gypsy moth found suitable and ample food; within 10 years, the population was completely stripping the leaves from large shade trees in residential areas surrounding the release site and alarming citizens of Medford (Medford Mercury Press 1906). The outcry and devastation prompted State and local authorities to investigate the biology of the gypsy moth and methods of control. At the same time, experimental applications of insecticides were begun. In 1893, the desire to test candidate pesticides during the winter led to the first documented indoor rearing of the gypsy moth since its introduction. Eggs were hatched "early in the season by means of artificial heat," and larvae were maintained on lettuce until leaves of elm, apple, or willow could be obtained (Forbush and Fernald 1896). Larvae were confined in open trays by tanglefoot smeared around the edges. Temperature and humidity were main-

tained at "comfortable levels" within the limits of standard heating systems. These early attempts to maintain the gypsy moth in the laboratory for experimental purposes were hampered by its univoltine life cycle, and by the lack of appropriate food during the late winter when eggs could be hatched at room temperature. Nevertheless, this style of rearing continued until the 1950's with little modification.

The gypsy moth is univoltine; eggs are laid in light-brown seta-covered masses in July and August; embryonation is completed in about 6 weeks; after this, the apparently fully developed larva begins its diapause. Egg hatch occurs in late April or early May, usually in synchrony with budbreak of the red oak, *Quercus rubra* L., the gypsy moth's major host in the northeastern United States. Males have five larval instars, and females have six; they range in size from about 3.5 mm in the first stage to about 30 mm in the sixth. Pupation begins in June and continues through late July. Adults eclose during July and August. Besides the limitations of the univoltine life cycle and diet, the large larval size and the long 35- to 40-day development period have been major obstacles to the development of a biologically sound and economical rearing methodology.

Between 1957 and 1960, intensive studies to develop laboratory techniques for rearing large numbers of gypsy moths were carried out at the U.S. Forest Service's Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory, New Haven, Conn. (Lewis 1961). Experiments were directed at determining the physical and biological factors affecting the growth and survival of the gypsy moth. The tests were conducted in walk-in environmental chambers built specifically to meet test requirements. Nutrition was supplied by plants grown in a greenhouse. As in the past, intraspecific variation in host-plant quality placed limits on interpretation of results involving insect growth and survival. Nevertheless, the tests provided direction for later studies.

Rachel Carson's book "Silent Spring," published in 1962, significantly affected forest-insect research and increased the demand for research on biological control, experimental diets, and a predictable means for rearing the gypsy moth. At first, attempts were made to maximize foliage quality by applying solutions of rice polish, sugars, and

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vitamins directly to the leaves. Although larval growth and survival apparently increased, off-season rearing remained unpredictable.

Rollinson (1964) began the first attempt to develop a uniform diet by modifying a wheat germ diet originally developed for the spruce budworm (McMorran and Grisdale 1963). This research ultimately led to the development of a diet and rearing technique (ODell and Rollinson 1966) that, with slight modification, is still being used for small-scale laboratory rearing. At the same time, a diet was developed at the Connecticut Agricultural Experiment Station, New Haven (Leonard and Doane 1966), and was used in studies on the development and behavior of the gypsy moth (reviewed by Leonard 1974). Other diet formulations for rearing the gypsy moth were later developed by Magnoler (1970), Vasiljevic and Injac (1971), and Ridet (1972).

In 1965, the U.S. Animal and Plant Health Inspection Service (APHIS) Gypsy Moth Methods Development Laboratory (GMMDL) at Otis Air National Guard Base, Mass., began studying ways to develop large-scale rearing of the gypsy moth to provide insects for research on the sterile-male technique, pheromones, the trapping of male moths for survey and detection, parasite production, and new insecticides. Several improvements were made (Secrest and Collier 1967, Tardif 1975), but reducing rearing costs was emphasized, and little attention was given to product quality. Although procedures to prevent microbial contamination were instituted, no internal standards were developed for measuring colony quality.

In 1975, with support from the U.S. Department of Agriculture's (USDA) Expanded Gypsy Moth Program, a USDA interagency team began investigating how to improve mass-rearing technology for the gypsy moth. The main stimulus for developing a large-scale rearing capability at that time was the potential of a virus specific to the gypsy moth; this virus is now registered under the name GYPCHEK. The goal of the team was to develop the best possible system for mass rearing, specifically: a low-cost diet and containerization, methods for handling insects, procedures for control of diseases, the best possible rearing environment, quality-control procedures, procedures for controlling the health and safety of workers, and pilot-scale facilities for insect and virus production. The accomplishments of this program through 1977 have been reviewed by Bell et al. (1981). The rapid improvement of the mass-rearing capability by 1977 was directly responsible for reevaluation of the sterile-male technique. It has also been largely responsible for the success of several other studies that require large numbers of uniform, healthy insects. Because this operation is so important to the pest-management program for gypsy moths, this paper will concentrate on

describing production, use, and quality testing now done at the APHIS GMMDL.

Colony Establishment and Maintenance

Facility

The facility began as several connected mobile homes in a large brick warehouse; these have been replaced by specially designed units built in the same space. The virus-production facility is next to and connected with the rearing area; traffic is routed for maximum isolation. Research laboratories, walk-in environmental chambers, a well-equipped shop, a large general work area, and offices are located next to the central rearing area. The rearing system is a prototype for facilities that mass-rear gypsy moths; so it functions mainly as a methods-development laboratory. At peak operation, the system is capable of producing about 25,000 insects a day.

Colony selection

Colony selection began 112 years ago when the gypsy moth was first introduced from France. The present North American strain was derived from the relatively few individuals that escaped in Medford, Mass. According to Mayr's founder effect (Mayr 1942), the initial laboratory stock would have been derived from a small amount of field material that was probably not genetically identical to the parent French population and contained only a small part of the original genetic variability.

In 1975, when the intensive research began on rearing of gypsy moths, several laboratory and wild strains were evaluated as potential breeding material for a standard colony. The evaluation considered the rate of population increase, the incidence of disease, and the rate of development. The laboratory strain selected and currently maintained as the standard colony was originally colonized from eggs collected in the winter of 1967 in an area near Blairstown, N.J. At that time, eggs were collected because there were many easily collectible egg masses; the collection was not intended as a starter colony for future production and was therefore not evaluated as potential breeding material. The decision to continue the strain was made on the basis of the question: "Why not?" The egg masses were relatively small, and many of the neonates died, apparently from virus. Disease, which was present from the beginning, continued to destroy part of each generation despite rigorous attempts to maintain and culture under sterile conditions. Resistance to disease was probably the strongest selective pressure on the colony. Researchers saved about 5% of each generation for colony production. The largest and most lively pupae

were chosen by eye; these characteristics were also used in selecting adults for mating. Eventually (generation not known), one could expect viable egg masses from 80% of the mated colony females; the starter colony had only 25%–40%. The decision to use the NJSS (New Jersey standard strain) as the standard was dictated by the evaluation procedure (and the colony's apparent improvement) and by the fact that this particular strain had been used by APHIS cooperators for many years. The decision was based on the strain's present performance not its past poor quality.

Colony maintenance and quality control

Techniques for colony maintenance and quality control are still developing as rearing procedures and sampling techniques are improved. In the system now in use, 10,000 neonates are implanted on the diet each week to maintain the colony and to accommodate emergency requests from cooperators. Neonates are transferred to the diet by hand with a soft camel's-hair brush. Even though this part of the rearing operation is not mechanized, there has been no problem in meeting colony or production requirements; an average worker can transfer about 1,500 neonates per hour. Rearing containers (XE-6, Sweetheart Plastics, Wilmington, Mass.), which contain 80 ml of a diet high in wheat germ (see table 1 for recipe) and eight neonates, are placed on plastic bread trays on aluminum carts. Each cart has 14 shelves holding 28 trays and 840 containers. The 5-inch space between the top of the container and the tray provides critical air circulation.

Airflow in the rearing room is distributed downward in a vertical laminar-flow pattern with a complete clean-air exchange—HEPA (high-efficiency particulate air) filtered, 95% DOP (dioctyl phthalate aerosol penetration) rating—every 3–5 minutes. At present, colony insects are reared in the same room as production insects; temperature is maintained at 25° C; relative humidity at 55%–60%; and the photoperiod at 16 hours of light, 8 hours of dark, with photophase beginning at 0500 hours. Environmental conditions in the rearing room are monitored continually by a wet-dry bulb recorder read from outside the room and by hygrothermographs located strategically in the room. Recorders are checked once daily to make sure that equipment is functioning properly. This monitoring helps to keep systems running smoothly and to minimize expensive equipment repair.

On artificial diet, development from newly hatched larva to pupa takes about 30 days (male, 29–30 days; female, 30–31 days). Containers are sampled at 7, 14, and 21 days postimplant date (PID) for synchrony of development and for survival. These measurements are compared on graphs to monitor colony performance, and deviations

Table 1.—Composition of diet used for rearing gypsy moths¹

Ingredient	Source	Grams per liter
Wheat germ, raw	Mennel Milling Co. Fostoria, Ohio 44820.	120
Casein	New Zealand Milk Products Rosemont, Ill. 60013.	25
Salt mix, Wesson.	ICN Biochemicals, Cleveland, Ohio 44128.	8
Sorbic acid	Dirigo Corporation South Boston, Mass. 02127.	2
Methylparaben (methyl p-hydroxy- benzoate).	Tenneco Chemical Piscataway, N.J. 08854.	1
Vitamin premix No. 26862.	Roche Chemicals Division of Hoffman-LaRoche Nutley, N.J. 07110.	10
Agar ²	Moorehead and Co.	15
Water		800

¹In 1980, the cost per liter=\$0.33; with HWG=\$10.24.

²Gelcarin HWG (Marine Colloids) at 1% (10 g/liter) may be used as an agar substitute.

from normal patterns are reported to the insectary management for investigation. Pupae are harvested at day 35 PIC, when about 80% of the insects have pupated. Two hundred each of the largest male and female pupae are selected by eye for colony production. These are segregated by sex in 16-ounce, unwaxed Dixie cups (50 males or 25 females per cup) and held in the rearing chamber until eclosion 10–12 days later.

The newly eclosed males and females (25 of each per carton) are placed in unwaxed ice-cream cartons lined with a layer of brown wrapping paper. Mating and oviposition usually occur within 24 hours; but, to insure completion of egg deposition, egg masses are not harvested for 72 hours. To allow embryonation, egg masses are held 25 days in the rearing room (25° C) before being transferred to a walk-in refrigerator. In refrigeration, they are maintained at 6° C, 90% relative humidity, and a 16 : 8 (hour) light : dark cycle for 120–180 days.

Colony performance for each implantation group (all containers in which neonates were implanted on any particular day) is measured in survival from neonate to pupa, sex ratio, mean weight (by sex) of 50 pupae harvested at day 35 PID, proportion of adults eclosing, and incidence and degree of adult deformity. Fecundity, embryonation, and hatchability are primary indexes for determining the rate of increase as a measure of population performance. Measurements include mean egg-mass weight, mean eggs

per mass, percentage of embryonation, and percentage of hatch. Standards are being established as sampling techniques are refined. Current sampling methods are relatively crude but allow for batch-to-batch comparisons—sustained change from normal fluctuations of any particular measure indicates a possible breakdown in the rearing system and requires investigation.

Thirty egg masses (about 700 eggs per mass) are removed from cold storage each week for colony production. Egg masses are disinfected, intact, in a 10% Formalin (formaldehyde) solution (see Shapiro 1977). Compared to a cleaning of dehaired egg masses with sodium hypochlorite, treatment of intact egg masses with Formalin significantly reduced egg mortality and the incidence of virus in young larvae (ODell and Rollinson 1966). After the Formalin treatment, the intact egg masses are placed in 100- by 25-mm plastic petri dishes, three masses per dish, and held in a controlled-humidity cabinet at 80% relative humidity and 25° C. Under these conditions, eclosion of eggs chilled for 180 days begins after the second day of incubation and continues for 3 days. Neonates are placed on the artificial diet after most of the population has hatched. In this procedure, larvae begin feeding quickly, and a 1 : 1 sex ratio is usually obtained at pupal harvest.

Recent investigations have focused on identifying critical factors in the rearing system that affect variation in insect development, size, and survival: once identified, these critical factors can be manipulated to stabilize normal variation, which hinders program objectives. Critical factors are also good places to start searching for causes of unsatisfactory colony performance. Some examples of critical factors and their treatment follow.

1. Elimination of microbial and fungal contamination significantly reduces mortality and enhances growth and synchrony of development. Primary treatment is the disinfection of eggs with Formalin. Additional treatment includes augmentation of the diet with microbial-inhibiting additives (as is done in the diet shown in table 1); provision of shoe covers and lab coats for personnel; disinfection of work surfaces, carts, and trays with Oxine (Biocide Chemical Company, Norman, Okla.); and daily misting of the main insect-handling room with Oxine. Airborne contaminants are monitored by standard agar plates (Trypticase soy agar for bacteria and Sabouroud dextrose agar for fungus). These are placed, 1 day per week, in each room of the rearing facility, and daily in the larval implantation room while neonates are being placed on the diet. Plates are incubated for 2 days before being checked. If bacteria or fungal colonies appear, appropriate steps are taken to determine the inoculum source. Colony and production insects that may have been exposed

are monitored more closely; significant increases in diet contamination or an increase in larval mortality may require that all insects from that implantation date be destroyed.

2. Regulation of moisture in the rearing container is critical to maintaining the best possible environment for the larvae. And excessive moisture will increase frass wetness, providing an excellent substrate for microbial growth and creating a mat that at times inhibits larval feeding. Treatment includes careful weighing and measuring of diet ingredients, precision in diet preparation time and in the volume of diet dispensed, and implantation of larvae within 2 to 4 hours after diet preparation. Variation in the volume of diet dispensed is monitored by randomly selecting and weighing five containers per day. Moisture is controlled mainly by having a container that provides adequate ventilation though only the lid is permeable. This container regulates respiration, rate of food depletion, and the amount of accumulated frass by controlling insect density—ideally eight larvae per container. Having appropriate humidity control in the holding room and maintaining an airflow across the top of the container are also essential.
3. Subtle changes in the quality of diet ingredients may be expressed by variation in rate and synchrony of larval development, pupal weight, and insect survival. To minimize such variation, 52 batches of diet ingredients are weighed and measured from the same stock of fresh ingredients and then stored at -15° C for use in colony maintenance throughout the year.

Personnel health and safety

The gypsy moth is notorious for its urticating setae and abundance of scales. Etkind (1976) tested the allergenicity of the gypsy moth and found that the antigens of cast larval skins and whole larvae caused positive immune reactions. Egg-mass hairs, though eliciting a significantly lower immune reaction, are easily airborne and cause sneezing and itching. These observations suggest that egg-mass hairs are a strong primary irritant. The main reason for installing HEPA filters in all units of the APHIS GMMDL rearing facility was to provide continuous cleaning of the air to remove insect hairs and scales and so reduce potential health problems associated with handling large numbers of gypsy moths. Also, work stations are equipped with exhaust hoods and vacuum systems to remove airborne hairs and scales and cast skins. In areas where adult moths and egg masses are handled, work stations are equipped with high-velocity exhaust ducts to remove the irritating scales and egg-mass hairs. Workers routinely wear protective clothing and gauze masks or respirators to further reduce poten-

tial health problems. A centrally located safety station is equipped with first-aid equipment for emergency and routine health care. And a regularly checked bulletin board at the safety station has space for management and staff safety suggestions.

Production and Use

Although procedures for production and colony maintenance are similar, production quality necessarily deviates from colony quality as levels of production increase. For example, at peak production in 1979, the amount of dispensed diet per cup varied by as much as $\pm 15\%$. The asynchrony in larval development caused by such variation was compensated for by increasing production and changing the time of pupal harvest. How the product will be used may allow other changes in the rearing system without significantly reducing product quality. For example, larval density during production for virus can be maintained at 10 per container (instead of 8) because deaths occur before the end of the feeding period and before food and available space are depleted. General procedures have been established for monitoring and controlling quality during production. For each specific use of the gypsy moth, quality traits are being identified, procedures implemented during insect development to monitor insect performance, and procedures established for systematically investigating the rearing system to determine causes for any deviation from standards.

Virus production

The nuclear polyhedrosis virus of the gypsy moth is host specific, naturally occurring, and a main cause of death in the gypsy moth during its outbreaks. In the laboratory, the nuclear polyhedrosis virus is produced from laboratory-reared larvae that have been hatched from disinfected eggs and reared in sanitary conditions on an artificial diet (Lewis 1976). In 1979, after 5 years of intensive investigation, laboratory-produced nuclear polyhedrosis virus was registered by the U.S. Forest Service under the trade name GYPCHEK.

Safety guidelines established by the U.S. Environmental Protection Agency (1975) for virus preparations for control of insects directly affect the production and quality control of the insects to be used for virus production. The guidelines are: "The fungal and bacterial contamination of the technical virus due to production procedures must not exceed the limits which can be maintained under sanitary manufacturing conditions. More importantly, human and mammalian pathogens (e.g. *Salmonella*, *Shigella*, and *Vibrio*) and microorganisms producing toxins known to affect mammals must be absent from the technical products."

The degree of infectivity of the nuclear polyhedrosis virus varies with the number of viable rods per polyhedra. The activity of the nuclear polyhedrosis virus product is calculated by a standard bioassay procedure that is part of the registration package for GYPCHEK (Lewis and Rollinson 1978) and must be within $\pm 20\%$ of the limit described in the standard bioassay. The NJSS was first used for virus production in 1977-78 when about 5,000 acre-equivalents (quantity required to spray 1 acre) were produced. During the winter of 1978-79, about 9,000 acre-equivalents were produced in the first attempt to mass-rear gypsy moths for virus production.

Quality control during rearing of insects for virus production.—Rearing of moths for virus production is similar to rearing for colony maintenance except that 10 larvae are put in each cup instead of 8. A standard minimum weight range for female larvae is established for 18 days PID, the time of virus challenge. The standard is based on previous production weights that produced maximum yields of polyhedral inclusion bodies per gram and low bacterial counts. Weights below the standard indicate a problem in the production system and initiate investigation. But production continues unless bacteria or fungal counts exceed standards or unless the diet quality has changed. Sanitary conditions are monitored constantly to insure that fungal and bacterial contamination is minimal.

Quality control during virus production.—At 18 days PID, larvae are transferred from the main production facility to the virus production area. They are then handled by separate personnel to minimize contamination of the production facility. After the virus is sprayed on the diet in each rearing container, all insects are transferred to an environmental chamber maintained at 29° C. Fourteen days after inoculation, the diseased larvae are frozen for 24 hours at -15° C. The frozen larvae are then freeze-dried, dehaired, and ground. The processed product is tested for quality by the following measures: (1) the number of polyhedral inclusion bodies per gram is determined for each implantation date; (2) bacterial and fungal contamination is determined by procedures described by Podgwaite and Bruen (1978); (3) each batch is bioassayed and the data evaluated by Berkson's logit chi-square method (Paschke et al. 1968); and (4) each batch is intraperitoneally injected into 10 white mice. If any of the mice die, the lot is rejected because the batch is contaminated with organisms not detectable by normal means. This is the primary quality-control test.

When contamination reduces virus quality, standard microbial techniques are used to identify and eliminate the source, and procedures are implemented to reduce re-establishment. Methods are being developed for investigating reduction in quality due to insect size and sus-

ceptibility to nuclear polyhedrosis virus. Presently, data derived from production control are analyzed for signs of breakdown in the system. The analysis determines whether further investigation is warranted.

Factors affecting virus quality.—Human error, which enhances microbial and fungal contamination during production, virus challenge, and harvest, is the main cause of poor virus quality. Airborne contamination caused by poor sanitary conditions is the result of human error, as are poor harvesting conditions, which increase microbial and fungal contamination in dead insects. Deviation from production procedures is likely to damage virus quality. For example, increasing insect density per cup or changing environmental conditions would likely increase variation in insect development and decrease polyhedral inclusion bodies per gram; a change in diet composition (for example, a reduction in vitamin C concentration) might increase insect susceptibility to bacterial contamination, producing more contamination in the final product. Quality of the virus may be measured indirectly by gypsy moth susceptibility. The degree of susceptibility to NJSS-produced virus depends on the geographic distribution, age, and physiological condition of the host, species of the host's food plant, and climatic conditions.

Sterile-male production

Use of the sterile-male technique for the gypsy moth was proposed as early as 1952 by P. A. Godwin. Studies of how gamma irradiation and chemical sterilants affect the gypsy moth male began in 1957 and continued sporadically through 1978 (reviewed by Mastro et al. 1980). Although gamma irradiation effectively sterilizes the adult male, implementation of the sterile-male technique for gypsy moth was inhibited by the inability to rear large numbers of the insect. At first, Richerson and Cameron (1974) and Richerson (1976) reported that certain laboratory-reared gypsy moth males, irradiated and non-irradiated, did not fly as well and were not as sexually aggressive as wild males. As these investigations used adults reared from both field-collected and NJSS eggs (generation unknown), it is difficult to speculate on the factors that contributed to the moth's apparent debility.

In 1976, because the gypsy moth rearing capability at the APHIS GMMDL had been improved (Bell et al. 1981; LaChance 1976), the USDA Expanded Gypsy Moth Research and Development Program supported new investigations of the sterile-male technique. These studies were to find out whether sterilized males could be used to suppress and eliminate newly established or isolated low-density populations of gypsy moth. First, the competitiveness of the nonirradiated NJSS adult male was evaluated. Then, the effects of irradiation and use—packaging, shipping, and distribution—were to be evaluated to pre-

vent confusion about the causes of any debility. Several operations (colonization, production, irradiation, and use) that might individually or collectively affect performance have been or are currently being evaluated. To date, only the effects of colonization and production have been adequately studied.

LaChance (1976) identified four performance factors relevant to the mass-reared gypsy moth's ability to compete with wild males: the ability to locate females in various situations and densities, the distance of dispersion, spatial and temporal activity patterns, and mating success. These factors are being used to assess the competitiveness of the NJSS male. They are also being used to establish performance indices based on measurements that can be done directly, in the field and in the laboratory. (See also Chambers 1977 for a discussion of general problems of quality control in mass rearing and for a review of behavioral deficiencies often found in programs for sterile-insect release.)

Quality control during production of insects for sterile-male release.—Insects being reared for sterilization are sampled at 31 days PID to determine percentage of pupation and synchrony of larval development. An indication that normal variation of either is being exceeded initiates investigation and allows advance warning to field crews applying the sterile-male technique that the production schedule may not be met. Abnormal performance also means that adults should be retested (see below) at the same time that more sterile males are released.

Quality control of adults in sterile-male production.—To assess competitiveness, tests requiring field release and recapture are conducted in forest areas with traps baited either with the pheromone (+)-disparlure⁵ or with females. The traps are arrayed vertically and horizontally in 70- and 140-m-radius circles around a central release point. The release point and trap sites are monitored hourly or continually. This monitoring provides quantitative data on periodicity of eclosion and dispersal, on spatial and temporal activity, on the ability to find mates at various sites, and on the ability and propensity to disperse. Data on survival and age of captured insects are obtained by monitoring trap sites for several days after release.

Onsite meteorological equipment and nearby weather stations provide data for establishing field-performance indices based on average temperature, humidity, and windspeed. Performance indices are also being used to evaluate laboratory techniques for testing quality; for ex-

⁵*cis*-7,8-Epoxy-2-methyloctadecane.

ample, demonstrating that a trait behaves the same way in both natural and controlled environments would indicate that direct measurements of that trait could be made in the laboratory.

At the APHIS GMMDL, quality testing is conducted with an actograph and flight tunnel (modifications of those described by Leppla and Spangler 1971—actograph—and Carde and Hagaman 1979—flight tunnel). Waldvogel (1980) found that the NJSS (F_{16}) preflight and in-flight response to (+)—disparlure was similar to that of wild males. The flight tunnel is now being used to profile preflight behavior in the presence of various concentrations of (+)—disparlure and at several temperatures. Field observations and measurements of preflight behavior of individual moths (NJSS and wild) and their mating success will determine whether flight-tunnel preflight measurements can be used to evaluate competitiveness as a mark of quality. The flight tunnel is also being used to characterize how pheromones affect behavior of males exposed as pupae or adults to various holding procedures during or after production. This information will provide more quality standards for analysis of the critical factors.

In 1979, researchers began actograph analysis to characterize the flight periodicity of NJSS (F_{16}) and wild males. With lights on and off simulating natural timing of sunrise and sunset and with constant temperature, humidity, and air movement, preliminary tests with the actograph indicate the same flight periodicity demonstrated in field release-recapture tests. With this success, the actograph is being used to establish performance indices for flight periodicity at different temperatures, humidities, and photoperiods. These performance indices will allow a laboratory comparison of various laboratory-reared and wild strains that can then be used to predict field performance and provide the standards for discerning critical factors affecting flight periodicity.

Factors affecting adult quality in sterile-male production.

In 1977, 1978, and 1979, field tests of wild and NJSS (F_{16} , F_{17} , and F_{18}) males demonstrated comparable performance in ability to locate females in various places in low-density populations (a simulation of 5–10 females per hectare), ability to disperse from the release point, and in mating success (Mastro and ODell 1978). But peak capture of NJSS males occurred about 2 hours later in the day than that of their wild counterparts. Actograph analysis of flight periodicity showed a similar asynchrony of activity. Similar asynchronies between laboratory-reared and wild male moths have inhibited other programs using the sterile-male technique (Raulston et al. 1976, White et al. 1977). So, such behavior is a defect in the NJSS male. Actograph studies are currently being conducted to iden-

tify colonizing or production factors that hamper periodicity of activity.

Parasite production

Since 1906, about 37 species of parasites of the gypsy moth have been introduced into North America, but only 10 species have become established (Hoy 1976). In evaluating possible reasons for establishment or non-establishment, Hoy (1976) discussed briefly the use of laboratory propagation of parasites for inoculative release and observed that "the practical problems of preventing genetic deterioration during laboratory propagation have . . . not been solved." Today, it is still true that, though laboratory research of behavior of gypsy moth parasites is increasing, the "practical problems" of laboratory propagation remain relatively untouched and unresolved.

Mackauer (1976) and Chambers (1977) have reviewed how laboratory production affects parasite performance; they found that adaptability and reproductive capability are primary measures of the quality necessary for the success of programs to release entomophagous insects. But little information is available on the biology and behavior of these parasites in their natural environments. Lack of such information limits characterizations of gypsy moth parasites.

Quality control in parasite production.—The NJSS is being used for production of gypsy moth parasites by the New Jersey Department of Agriculture in Trenton, the Connecticut Agricultural Experiment Station in Hamden, the University of Massachusetts in Amherst, and the Forest Insect and Disease Laboratory also in Hamden, Conn. Each of these organizations has a unique rearing system, so comparing their production methods and products is difficult. But they all have problems with periodic, sometimes disastrous contamination by bacteria and fungi. Gypsy moth eggs are sterilized, and work surfaces and equipment are routinely cleaned at each facility; but inadequate environmental control, other facility peculiarities, and the additional stress of parasitization make control of diseases and fungi extremely difficult.

Each year, the New Jersey Department of Agriculture produces about 2.5 million gypsy moths to use at various life stages for parasite production. The NJSS is used for about 30% of the production; the rest is produced from gypsy moths reared from egg masses collected each year from natural populations. Larval mortality and variation in the rate of larval development preclude the use of about 50% of all larvae hatched. Most of this loss is caused by contamination, poor vigor, and the intrinsic variation in field-collected populations. Although no specific data are available, more parasites are produced

from NJSS larvae than from larvae reared from field-collected eggs.

In New Jersey, the gypsy moth is reared on one of three diets, depending on which one most increases numbers of parasites for the least money. For example, *Rogas lymantriae* Watanabe is produced in hosts reared on a modification of the diet described by ODell and Rollinson (1966). The less expensive but more nutritious (for the host—R. A. Bell, personal communication) high wheat germ and modified hornworm diets (both produced by BioServ, Trenton, N.J.) have been tried. But in both cases, hosts die soon after oviposition by *R. lymantriae*, without producing a parasite. The causes of the apparent differences in survival of host and parasite are unknown and are not now being investigated (R. Chianese, personal communication).

The NJSS are shipped as pupae from the APHIS GM-MDL to the Connecticut Agricultural Experiment Station and to the Entomology Department at the University of Massachusetts for production and study of *Brachymeria* spp., pupal parasites of the gypsy moth. The use of hosts reared in the same system (APHIS GMMDL) reduces probable variation in performance, size, reproduction, success, survival and behavior, caused by different methods of host rearing; so the two studies are complementary (though the *Brachymeria* populations will have intrinsic differences).

The Connecticut Agricultural Experiment Station also uses NJSS larvae, reared from eggs on the modified hornworm diet, for producing *Apanteles melanoscelus* Ratzeburg (R. Weseloh, personal communication). NJSS is preferred over wild strains because there is significantly less mortality of parasitized hosts. But comparisons in size, reproduction, success, survival, and behavior have not been made between parasites produced on various host strains (R. Weseloh, personal communication).

The Forest Insect and Disease Laboratory at New Haven uses NJSS to produce several species of gypsy-moth parasites for research. *R. lymantriae* is being investigated to determine whether it can be established in natural habitats. Adult and mummy weights, fecundity, and mating behavior are being recorded to establish indices of colony performance. Environmental conditions are being monitored closely so that changes in behavior and in population characteristics can be analyzed (W. E. Wallner, personal communication).

Factors affecting parasite quality.—Specific changes in behavior and in population characteristics in parasites and other insects subjected to laboratory colonization (Boller 1972, Mackauer 1976, Chambers 1977) have not been reported for parasites reared on NJSS. ODell and

Godwin (1979) demonstrated that NJSS could be used to rear large numbers of *Blepharipa pratensis* Meigen. In a concurrent study (unpublished), they found that the puparial weight of two groups of *B. pratensis*—reared at the same time, under the same environmental conditions, and on hosts fed the same diet (ODell and Rollinson 1966), but on different gypsy moth strains, differed significantly from each other. The difference depended on the geographic location and the density of the population that the host eggs were collected from. The puparial weights of *B. pratensis* reared from NJSS were similar to those of parasites reared on hosts from low-density populations.

A few studies have been done of parasite rearing on gypsy moths other than the NJSS. For example, Shapiro (1956) found that *B. pratensis* (= *Sturmia scutellata* Robineau-Desvoidy) was heavier when it emerged from gypsy moth from low-density populations. Greenblatt and Barbosa (1980) had similar results with *Coccygomimus turionellae* (L.), but they had different results with *Brachymeria intermedia* (Nees), which were heavier when reared in hosts from high-density populations. At the Pennsylvania Biocontrol Laboratory (Bureau of Forestry, Division of Forest Pest Management, Harrisburg), the size and fecundity of adult parasites are monitored routinely to insure that the parasite colony maintains a specified degree of quality; generally, gypsy moth parasites reared on natural hosts are larger and produce more eggs than those, such as the greater wax moth, *Galleria mellonella* (Linnaeus), reared on unnatural hosts (R. A. Fusco, personal communication). Hoy (1975a, 1975b) pointed out the need for field evaluation of laboratory parasites, and for more observational studies on their behavior. Certainly, if standard laboratory strains such as NJSS are to be used for research and development, such studies are justified.

Production for research and development

Leppa (1979) summarized the value of laboratory-reared insects for research, noting that they are the “white rats” of the entomologist, being “uniform, relatively inexpensive, continuously available, and comparable to their counterparts in nature.” At this time, the NJSS is not a “white rat.” Bioassays and standards for detecting changes in the NJSS that might occur because of rearing modifications, microbial contamination, and genetic selection are just now being established. Nevertheless, NJSS is widely used for research and development.

In 1976, NJSS ($F_{1,4}$) larvae were evaluated for use in bioassaying plant extracts for deterrent compounds (Doskotch et al. 1977). Such use would allow NJSS to be used during the winter when larvae from field-collected eggs are not available. In the bioassay, the dry weight of

frass was used to estimate the amount of feeding and the relative detergency of various plant extracts. Third-stage larvae were reared from NJSS and from field-collected eggs by the method described by O'Dell and Rollinson (1966). When tested, the NJSS third-stage larvae consumed twice as much of the control diet (extract from red oaks) as was consumed by their wild counterparts; but there was no significant difference in the proportional decrease in feeding response to an added deterrent compound (extract from *Liriodendron tulipifera* L.). A later bioassay of other leaf extracts produced similar results; so we were confident that NJSS larvae could be used in the bioassay without compromising project objectives (T. M. O'Dell, unpublished data).

NJSS larvae reared from eggs on a modified O'Dell-Rollinson diet, where antibiotics were omitted, are used to bioassay formulations of *Bacillus thuringiensis* (Berliner). The bioassay compares the LD₅₀ of each test formulation with that of a universal *B. thuringiensis* standard (Dulmage et al. 1971). There are indications that NJSS larvae reared from eggs that have been held under refrigeration (4°–6° C) for extended periods of time (200 days or more) do not respond normally to the standard; that is, there is inconsistency in the normal variation of the standard LD₅₀. To avoid this abnormal response, new preparations are tested with larvae hatched from eggs held under refrigeration for 175–185 days.

NJSS larvae are also being used to study how secondary metabolites affect insect growth and survival and to screen insecticides. In each case, comparisons are being made with larvae reared from field-collected eggs (M. E. Montgomery and W. McLane, personal communications). NJSS adults are being used by researchers at Michigan State University, East Lansing, to characterize the locomotor activity and pheromone response of males in various field situations. NJSS adults are also being used by APHIS GMMDL scientists to evaluate pheromone-trap designs. These scientists (Michigan State and APHIS GMMDL) use the adult quality-control evaluations developed for NJSS sterile-male release.

Challenges

There have been many advances in production, use, and quality control of the NJSS since its establishment in the laboratory in 1967. But, in making these advances, we have learned how much more we need to know to improve the scientific production and use of the gypsy moth for biological control and research. The following is a list of the many challenges we still have facing us at the APHIS GMMDL.

1. Determine and maintain optimal storage conditions and shelf life for perishable dietary ingredients, par-

ticularly wheat germ, casein, vitamins, and gelling agents.

2. Standardize through automation the processing and dispensing of diet (mainly to eliminate day-to-day variation).
3. Automate implantation of eggs and larvae on diet, and automate harvesting of pupae.
4. Simplify monitoring of developmental and reproductive performance.
5. Determine how many insects can be successfully reared per unit of space if the rearing system is further refined.
6. Determine what environmental conditions will foster the best rate and synchrony of development (particularly for egg hatch, pupation, and adult eclosion).
7. Develop a method for rearing mostly males for sterile-male production and mostly females for virus production. (The female produces more virus because it is larger than the male.)
8. Develop and explore the use of nondiapauses or short-diapause genotypes to augment or replace the existing diapause strain (to reduce or eliminate the requirement for chilling and to shorten generation time).
9. Develop bioassays, and establish standards for evaluating insects for consistency of performance in tests for standard toxicity and activity.
10. Develop systematic methods for investigating deviations in production standards and deterioration in product quality.
11. Develop a communication system for informing users of production and product change, and encourage the establishment of internal standards for periodic evaluation of the product received.
12. Develop an antiserum for desensitizing personnel acutely allergic to gypsy moth setae and scales.
13. Investigate relative chilling effect of air movement on eggs being held at various temperatures (0°–7° C) and at 70%–100% relative humidity.

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Rearing the Tobacco Budworm, *Heliothis virescens*, and the Corn Earworm, *Heliothis zea*

By J. R. Raulston¹ and E. G. King²

Introduction

Scientists doing entomological research with *Heliothis* spp. have long recognized that laboratory colonies of these insects are an important research instrument. Ellisor (1935), having found himself without enough field-reared corn earworms, *Heliothis zea* (Boddie), to complete certain laboratory studies, devised a way to rear them in the greenhouse. His method required caging adult moths on young corn plants to lay eggs and then transferring third- and fourth-stage larvae to soaked sweet corn kernels for completion of larval development. Barber (1936) described a culturing method that required placing first-stage corn earworm larvae on the silk of corn ears that had been silking for less than 10 days. Barber found that larval development was completed in 2 to 3 weeks, development periods that corresponded with those observed in the field. These early attempts to culture corn earworm in the laboratory had drawbacks, and Stahler (1939) described a disease that decimated populations being reared on lettuce and alfalfa in the insectary. The disease symptoms described indicate that the larvae were dying from a nuclear polyhedrosis virus.

Methods similar to these were used for many years in propagating corn earworm in the laboratory. As recently as the early 1960's, Callahan (1962) described techniques and apparatus for rearing corn earworm larvae on corn and for enhancing mating and oviposition by adults. But he was unable to maintain the laboratory culture for more than five generations and concluded that the diet lacked certain ingredients needed for continuous culturing.

It became possible to rear *Heliothis* spp. under continuous laboratory culture divorced from its natural hosts after the nutritional requirements for various insect species were elucidated and artificial diets were developed (see reviews by House 1965 and Dadd 1970). Here, we

will review several subjects important to the laboratory propagation of *Heliothis* spp.—diet development and evaluation; diet preparation and handling; rearing equipment and techniques; facility design and flow patterns; and how pathology, physiology, and behavior affect quality control.

Diets

The first published attempt at rearing the corn earworm on an artificial diet was that of George et al. (1960) using a diet similar to one developed by Bottger (1942) and Beck et al. (1949) for the European corn borer, *Ostrinia nubilalis* (Hübner). George et al. (1960) were unable to rear the corn earworm through its complete larval cycle on their diet and had to feed the first-stage larvae on corn silk. Vanderzant et al. (1962a) were able to rear the corn earworm from egg to adult on a purified diet, and they determined a dietary requirement for ascorbic acid. Because this diet had so many ingredients, Vanderzant et al. (1962b) went on to rear the corn earworm on a wheat germ diet modified from one developed by Adkisson et al. (1960) for the pink bollworm. The wheat germ diet, which contained fewer ingredients than the previous diet, was adequate for normal larval development. Using as a base diet the one she and others reported earlier (1962a), Vanderzant (1968) later determined the lipid and sterol requirements of the corn earworm. She also determined that sterol, linoleic acid, linolenic acid, choline, and inositol were needed in the diet.

Based on these initial investigations and on the nutritional requirements established by Vanderzant and others, several diets, many only slightly modified from that reported by Vanderzant et al. (1962b), have been developed for rearing *Heliothis* spp. But Shorey (1963) developed a radically different diet based on lima beans and brewer's yeast for the cabbage looper, *Trichoplusia ni* (Hübner), and Shorey and Hale (1965) modified this diet slightly for rearing nine noctuid species including the corn earworm. Burton (1969) further modified the bean diet for rearing the corn earworm and tobacco budworm, *H. virescens* (Fabricius), by adding wheat germ and replacing brewer's yeast with the less expensive torula yeast. Patana (1969) used baby lima beans in the bean diet for rearing several lepidoptera, including the corn earworm and tobacco budworm and reported larvae fed more readily on this diet. Patana and McAda (1973) also

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made dry flakes from the bean diet for use as a physical barrier to separate feeding tobacco budworm larvae, which are cannibalistic in the later instars.

Most of the recent diet modifications for rearing *Heliothis* spp. have emphasized reducing cost. Burton (1970) reported the development of a diet for the corn earworm based on a child-food supplement, CSM (corn-soya-milk), and Burton and Perkins (1972) developed a diet for rearing corn earworm and tobacco budworm that is based on a blended human-food product, WSB (wheat-soya blend). Insect growth and development on these diets are comparable to those on the bean diet but cost about half as much. Perkins et al. (1973) made further comparisons of the bean, CSM, and WSB diets with another diet containing opaque-2 corn and concluded that the WSB is nutritionally and economically best for rearing *Heliothis* spp. Other researchers have modified the casein and wheat germ diet reported by Vanderzant et al. (1962b) to reduce its cost. For rearing tobacco budworm, (1971) Shaver and Raulston replaced casein with a toasted, defatted soyflour. Prepared with the agar-reduction method reported by Raulston and Shaver (1970), this diet has been about 50% cheaper than the casein-wheat germ diet. For corn earworm, Brewer et al. (1975) concluded that soyflour is better than cottonseed meal or corn germ flour as a source of protein. Brewer and Martin (1976) also reported using various sawdusts or corncob grits and found them to be an economical means of reducing the amount of agar used in the corn earworm's diet. Brewer and King (1979) reported that the corn earworm requires 3.4 g of the soyflour diet containing corncob grits to complete larval development and that the tobacco budworm needs 2.7 g. Brewer and Tidwell (1975) and Raulston (1975a) also evaluated the effect of reducing the B-vitamin supplement used in their diets and found it can be reduced by as much as 20% with no harmful effects. Generally, institutions or laboratories that maintain small laboratory colonies of *Heliothis* spp. still rely on the original casein and wheat germ diet reported by Vanderzant (1962b); however, most researchers doing large-scale rearing have been forced by high costs to develop new diets or modify existing ones.

Diet Preparation and Handling

Maintaining small laboratory colonies of *Heliothis* spp. requires little sophistication in diet preparation and dispensing. In fact, both are often done with methods first reported by Berger (1963), who prepared his diet in a small blender and dispensed it into rearing containers from squeeze bottles. Later, as demand increased for more production, researchers devised equipment to facilitate the preparation and handling of the diets. Burton et al. (1966), Patana (1967), and Stadelbacher and Brewer

(1974) reported dispensing diet from containers under pressure. Diet-preparation capacity was increased by the use of large blenders—for example, the 38-liter Hobart VCM 40E used by Burton (1969). And Raulston and Lingren (1972) reported use of a stainless steel tank capable of preparing 177 liters of diet. This system used an overhead Lightnen Mixer, Model NM-1 to mix the diet ingredients rapidly. Raulston and Lingren (1972) dispensed their diet with a peristaltic pump, but later they changed to a positive-displacement pump like the one described by Patana (1977). Harrell, Sparks, Hare, and Perkins (1974) described a mechanized system for mixing large quantities of dry ingredients (225 kg) and a continuous subsequent flow of flash-sterilized diet to a form-fill-seal machine modified for rearing corn earworm larvae. This system maximized production. Brewer (1977) agreed that flash sterilization of the corn earworm diet did not affect larval growth and development, but found that antimicrobials were still necessary in the diet because of recontamination.

Larval Rearing Methods

At first, larval-rearing techniques were labor-intensive and evolved from nutritional studies where only a few insects were handled. Glass vials plugged with cotton were commonly used by George et al. 1960; Brazzel et al. 1961; Vanderzant et al. 1962a, 1962b; Berger 1963; Chauthani and Adkisson 1965; and Vanderzant 1968). Diet was typically dispensed into the vial from squeeze bottles, and neonate larvae were placed on the gelled diet with a fine brush.

Automated methods for dispensing the diet and handling the vials were developed in response to increased demand (Burton et al. 1966). These vials required cleaning and sterilization for reuse; so disposable plastic cups were substituted for the vials. Burton and Cox (1966) and Burton and Harrell (1966) modified a foodpacking machine to dispense the cup, dispense diet into the cup, and cap the cup. Burton (1969) modified this procedure for corn earworm rearing, but the neonate larvae had to be placed in the cups by hand. Harrell et al. (1969) developed a machine that crushed the cups, so pupae could be harvested, then separated the pupae from the diet and crushed cups. Harrell et al. (1970) described a device to glue corn earworm eggs to the cup lid, thus eliminating the need for larval implantation. Use of these automated devices allows processing of about 3,600 cups per hour. Other nonautomated methods were developed that use plastic cups for rearing *Heliothis* spp. Patana (1969) described some of these procedures, which are often used for maintaining small laboratory colonies.

Shorey and Hale (1965) first used compartmentalization for rearing *Heliothis* spp. larvae by fitting cardboard

dividers into 175-ml cups. Compartmentalization became an accepted technique for rearing *Heliothis* spp. larvae after Ignoffo and Boening (1970) reported rearing larvae in compartmented disposable plastic trays covered with Mylar film. Ignoffo and Boening transferred neonate larvae into the containers with a brush before sealing the unit; or they used the larval transfer system described by Gard (1969) after the unit was sealed. E. A. Harrell and others (Harrell et al. 1973; Harrell, Sparks, Hare, and Perkins 1974; Harrell, Sparks, Perkins, and Hare 1974) automated procedures for using the compartmented trays for rearing corn earworm larvae by fitting an inline form-fill-seal machine (that formed the trays) to a device for dispensing diet and implanting eggs. Equipment was also devised to collect the pupae from the trays. Sparks and Harrell (1976) described the complete automated rearing system and reported that at top speed the system could process 261 of the 120-cell trays per 8-hour day.

Another system that diverged from the compartmentalized trays was the use of cells that could be placed into trays. Roberson and Noble (1968) first reported rearing tobacco budworm larvae in honeycomb-like cells formed from Mylar. Raulston and Lingren (1969) improved this technique and later developed a rearing cell from Polystyrene light-diffusion louvers that could be reused indefinitely (Raulston and Lingren 1972). These authors also devised a simple technique for implanting eggs into the cell unit. Other researchers have further modified the cell-rearing technique by replacing the top made of screen-polypropylene cloth and polyurethane foam described by Raulston and Lingren (1972) with a single sheet of 3.2-mm-thick polypropylene-Porex filter (Hartley et al. 1982). This unit is now being used to mass-produce the sterile backcross ($BCn\varnothing \times$ tobacco budworm σ) from the intercross [tobacco budworm $\varnothing \times H. subflexa$ (Guenée) σ]. About 4 million *BC* pupae have been produced by this technique over the last 3 years (1979–81); also in the late 1970's, during another pilot sterile-male release project involving tobacco budworm pupae, a production level of about 60,000 pupae per day was sustained for 4 years at a cost of less than \$5.00 per 1,000 pupae.

Adult Handling

Various container types have been used as oviposition cages for *Heliothis* moths. Two of the most common containers have been the 3.8-liter glass jar and the 3.8-liter cardboard ice-cream carton. About 10 pairs of moths can be maintained in these ice-cream cartons without affecting fecundity or fertility, but more (20 pairs per carton) reduce fertility (Burton 1969). In each oviposition container, the top is replaced with a piece of cloth, and cloth strips (streamers) are suspended from the container top's edge to the bottom. The cloth top and streamers serve as oviposition substrates. The moths are fed diluted honey

or a sucrose solution. Callahan (1962) used plywood cages measuring 25 by 25 by 30 cm as moth holding and oviposition cages. The cage front was fitted with a cloth or paper cover that served as an oviposition site. He concluded that four to six pairs of corn earworm moths are best for optimum oviposition in this size of cage. His studies showed that light, temperature, and humidity are critical elements in obtaining maximum mating and oviposition. In fact, no mating by corn earworm moths occurred when the moths were held at temperatures above 29.4° C.

Devices have been developed for continuous removal of *Heliothis* spp. moth scales because they are a human health hazard. Burton (1969) described methods for filtering scales from the work area while collecting oviposition cloths. Raulston and Lingren (1972) developed emergence cages for large-scale production that have an enclosed scale-filtering system and a device for transferring adults from emergence cages into the oviposition cages, minimizing handling.

Recently, Gross et al. (1975) and McWilliams et al. (1981) have developed techniques for managing the moths in large quantities with minimum handling. Gross et al. (1975) described a plywood box partitioned vertically by five waxed-paper sheets into four oviposition chambers. Each chamber was fitted with a continuous flow of beer as a diet source and provided oviposition sites for 100 pairs of adults. The waxed-paper sheets could be pulled through the bottom of the chamber and the eggs brushed off with a hog-bristle brush. McWilliams et al. (1981) reported using oviposition cages that also include devices for adult emergence and feeding. The cages are housed in a chamber that confine and collect the moth scales. The moths lay eggs on a standard aluminum-frame, fiber-glass-mesh screen that fits into grooves in the cage framework and can be easily inserted or removed. McWilliams has gotten sustained production of about 1.5 million eggs per week by operating 15 oviposition cages needing about 5,500 pupae per week. The operation requires about 15 hours of labor per week.

Facilities for Rearing *Heliothis*

The advances in dietary and technological developments facilitated the rearing of *Heliothis* spp. and allowed their continuous colonization in the laboratory. But many colonies still fail; Sparks and Harrell (1976) cited both the lack of facilities to maintain proper environment and the inability to eliminate massive disease outbreaks as the main causes of failing to increase production. So, however sophisticated rearing technology has become, large-scale, or even small-scale, production of *Heliothis* spp. will not be uniform unless facilities are properly designed for such

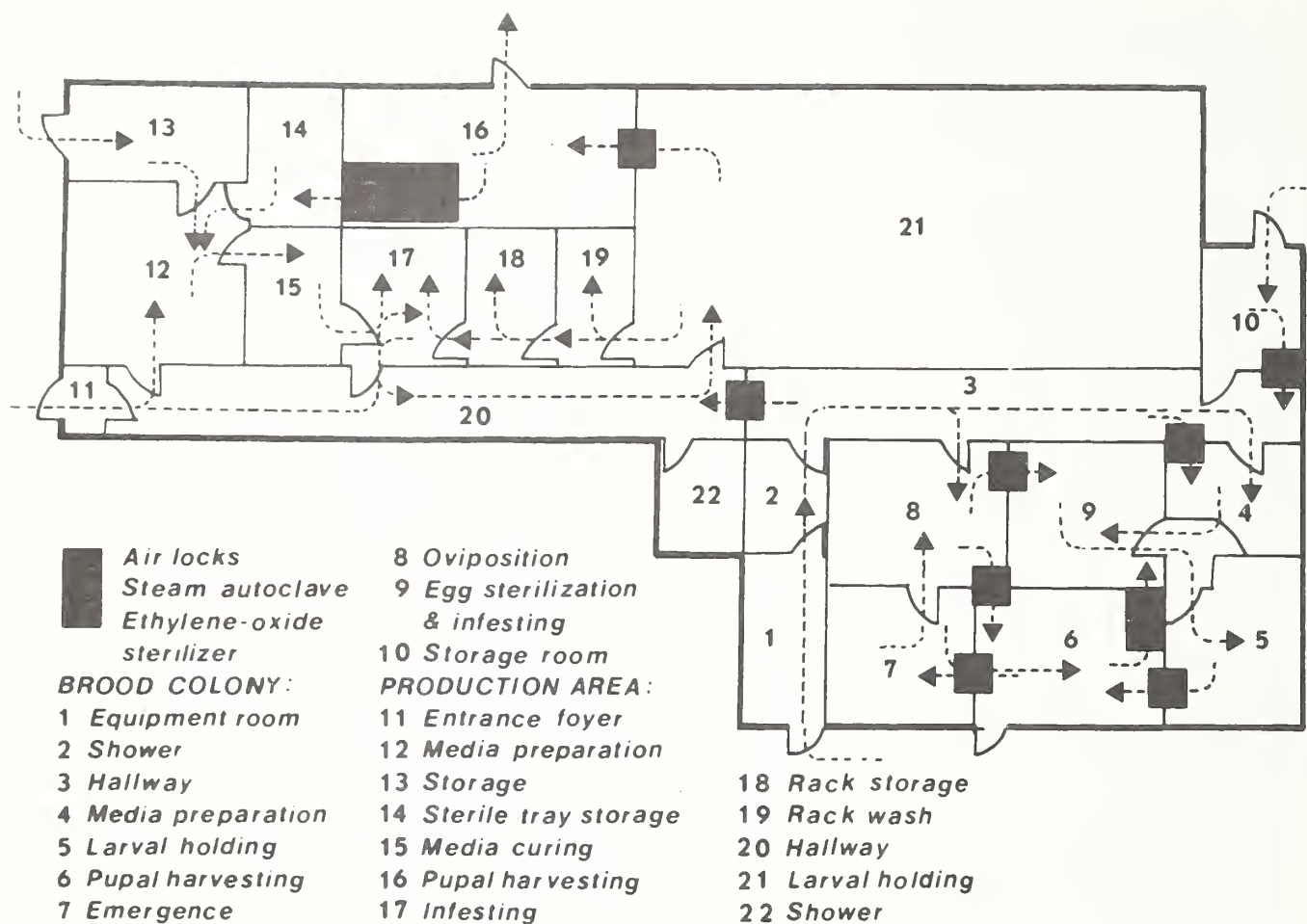


Figure 1.—Floor plan and flow pattern of the U.S. Agricultural Research Service's tobacco budworm rearing facility, Brownsville, Tex.

rearing. (See Chambers 1978, specifically, and Leppla and Ashley 1978, generally, for some of the requirements for such rearing facilities.) Here, we will concentrate on the design and operation of two facilities that have been successfully used for rearing tobacco budworm on a relatively large scale.

In the U.S. Agricultural Research Service's rearing facility at Brownsville, Tex., Raulston and Lingren (1972) used a chilled-water environment control and relatively unsophisticated conventional cleanroom technology as described by Austin and Timmerman (1965). Use of chilled-water environment control eliminated the transfer of air between rooms and allowed the environment in each room to be separately controlled. The system was designed so that all rooms, except the pupal-harvest room, were under positive pressure. All the recycling air and the fresh air being introduced into each room passed

over the conditioning coils and through a HEPA (high-efficiency particulate air) filter with a pore size of $0.3\ \mu\text{m}$ before entering the room. One-hour exposures of nutrient agar plates in each of seven rooms in the stock-culture area resulted in an average mold-colony growth of 0.16 per hour and an average bacterial-colony growth of 4.2 per hour. These data demonstrate how well the system reduced airborne contaminants.

The system (fig. 1) was designed as two separate and autonomous facilities comprising areas for stock culture and large-scale production. The only junction between the two areas was a passthrough box that allowed eggs to pass from the stock culture into the mass-production area. The one-way flow pattern was designed to move both personnel and materials through the system from clean to less-clean areas. The stock-culture area was maintained under strict quarantine, and personnel were re-

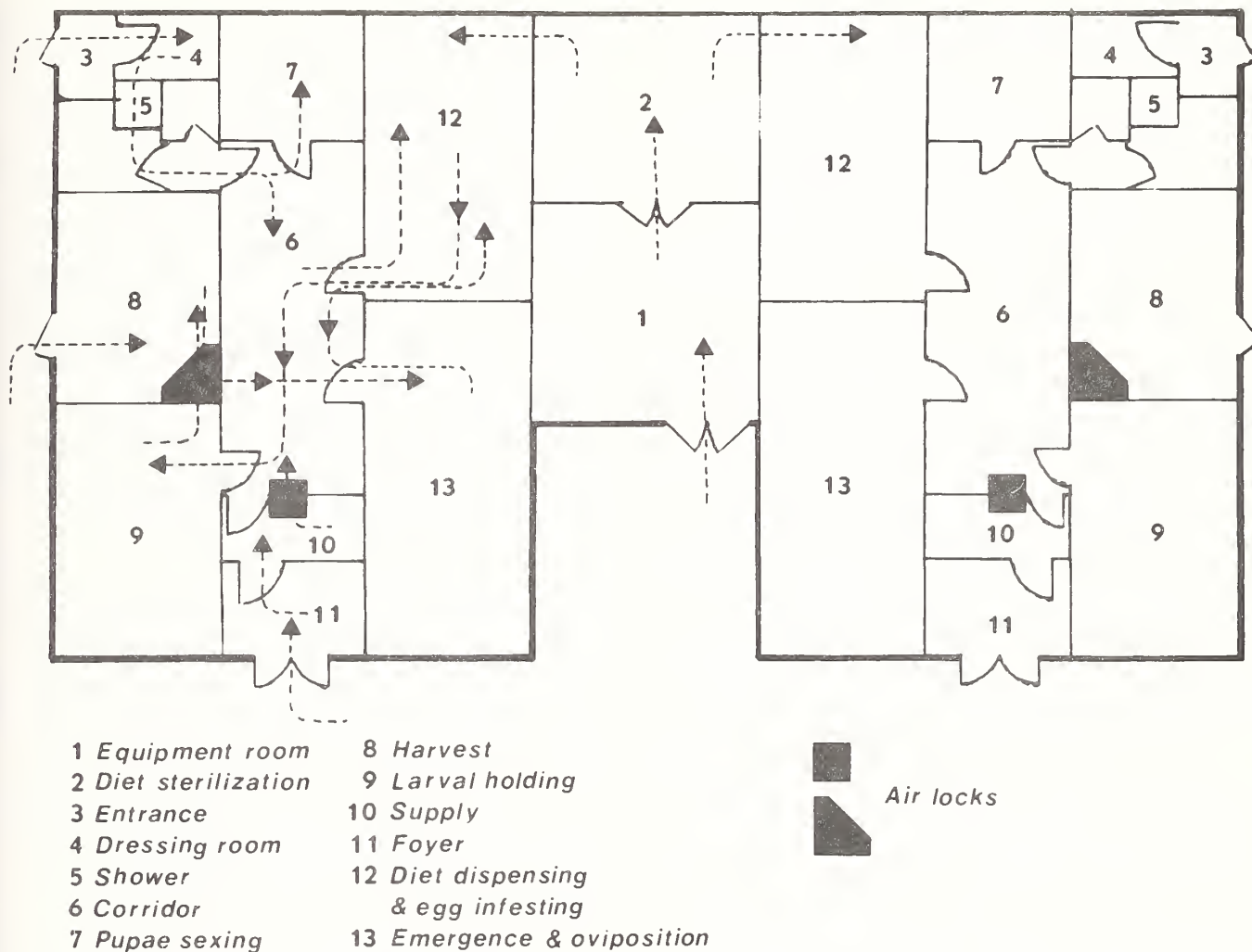


Figure 2.—Floor plan and flow pattern of brood-colony units for producing tobacco budworms and sterile backcrosses at the U.S. Agricultural Research Service's Delta States Research Center at Stoneville, Miss.

quired to shower and change into clean garments before entering the work area. The quarantine was placed on the stock-culture area because it was involved in culture propagation, and it was believed that only disease-free cultures would result in uniformly high quality. Since the production area produced only pupae for use outside the facility, no adult-handling facilities were incorporated into its design; however, rigid restrictions were still maintained on movement of personnel into the facility and through the work areas. The tobacco budworm culture introduced into this facility was thoroughly examined for nuclear polyhedrosis virus, cytoplasmic virus, and microsporidia before its introduction. The culture was monitored weekly, and the colony was kept free of

disease from the time of its introduction in 1969 until the facility was closed in 1977.

The second facility (fig. 2) was constructed for brood colonies to produce sterile *Heliothis* hybrids at the U.S. Agricultural Research Service's Delta States Research Center, Stoneville, Miss. (Brewer et al. 1978). This facility was also designed to use a chilled-water environment control and absolute air filtration for reducing microbial contaminants. It is maintained under positive pressure except for the harvest area, which is maintained under negative pressure. The two sides of the facility are mirror images. On one side, tobacco budworm has been produced for culture maintenance; that side has also been used to

produce sterile males to be used in the other half of the facility for backcrossing to F_n -progeny females for egg production. Mass-production of sterile hybrids has then been done in another facility. Located between the two halves of the facility is the diet-preparation area, which is not maintained as a cleanroom. After preparation, the diet is flash-sterilized and pumped through a closed system to the desired area. So a sterile diet kitchen is not needed. Colonies being reared are maintained under quarantine to prevent introduction of disease organisms.

Quality Control

The application of cleanroom techniques and the best available environment-control systems has greatly facilitated the production of *Heliothis* spp. in continuous closed cultures. These advances and the automated and highly efficient rearing procedures provide the technology for mass producing *Heliothis* spp. as needed. Such production efforts require that rigid standards be placed on the product (the insect) and that a monitoring system be established to insure that these standards be met. Several aspects of quality must be considered in the development of monitoring systems (see, for example Mackauer 1972, 1976; Huettel 1976; and Chambers 1977). Huettel (1976) lists the major components associated with insect propagation in the laboratory, their measurable traits, and possible methods for monitoring such traits.

Both corn earworm and tobacco budworm are susceptible to several diseases that can rapidly become epidemic in the confines of the laboratory. So one of the first needs of quality control is monitoring for disease organisms. Three of the major disease organisms associated with laboratory propagation of *Heliothis* spp. are nuclear polyhedrosis virus, cytoplasmic polyhedrosis virus, and the protozoan *Nosema heliothidis* (Lutz and Splendor). Outbreaks of nuclear polyhedrosis are normally self-limiting since those insects that contract the disease die. Both the cytoplasmic virus and the protozoan, however, are easily transmitted across generations—cytoplasmic virus on the egg's surface (Mery and Dulmage 1975) and *Nosema* in the egg (Brooks 1968). So these two diseases may cause a general decline of the laboratory culture with prolonged development, increased mortality, and decreased fecundity and fertility. A disease-monitoring system should include direct measurements on these physiological traits and autopsies of suspect individuals by a competent pathologist. The best method for handling disease in laboratory colonies is prevention. In fact, disease organisms in a colony are intolerable since the quality of the product can no longer be guaranteed.

Quality insect rearing is dictated by intended use of the product. So traits desirable for specific culture uses, as in a laboratory bioassay or in a hybrid or sterile-insect

release, may not be consistent and may be mutually exclusive. H. T. Dulmage (personal communication) lists desirable traits in a tobacco budworm culture used to bioassay *Bacillus thuringiensis* Berliner isolates; these are uniformity in larval growth, reproducibility of results over time, and the culture's capacity to reproduce enough insects to meet bioassay demands uniformly and predictably. These traits would describe a culture that had already undergone the dynamic period of laboratory adaptation, was genetically stable and disease free and was being reared on a uniform artificial diet under a uniform set of laboratory conditions. But traits that would be considered desirable in a culture of *Heliothis* spp. destined for use in a sterile-male program would be much different. Here, desirable traits would be ability to disperse within the ecosystem, ability to locate and use host plants, and ability to locate and compete for native virgin female mates. Maintenance of these characteristics would require developing a rearing regimen that would insure a wide genetic base and minimize genetic changes caused by laboratory adaptation.

Young et al. (1976) developed a crossing scheme to minimize inbreeding in a culture of corn earworm and concluded that the method improved colony performance in mating, fecundity, and fertility. No behavioral traits were measured in this investigation. Raulston (1975b) reported laboratory adaptation of a wild strain of tobacco budworm and found that selection pressure changes the mating and oviposition patterns beginning in the sixth and seventh generations. Crosses between the laboratory and wild strains showed that these changes are genetically controlled; however, a genetic analysis was not made. Raulston et al. (1976) and Lingren et al. (1979) further observed a mating asynchrony of about 2 hours between a long-established laboratory colony and wild tobacco budworms in field conditions. A similar asynchrony with a lack of mating interaction was observed when this same laboratory strain of tobacco budworm was used in producing backcross progeny from hybrids resulting from a cross between the tobacco budworm and *H. subflexa* (Raulston et al. 1979). But, when J. R. Raulston and A. N. Sparks (unpublished data) used trapped wild males in producing the backcross progeny (having the laboratory-colony background), this asynchrony and lack of mating interaction were no longer observed.

It is the responsibility of personnel involved in the development and production of *Heliothis* spp. to determine traits that must be retained or incorporated into the colony. Without this input, a scientifically acceptable product cannot be developed, regardless of our physical sophistication and production capability.

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Mass Rearing the Pink Bollworm, *Pectinophora gossypiella*

By Fred D. Stewart¹

Introduction

The main reason for mass rearing the pink bollworm, *Pectinophora gossypiella* (Saunders), at the U.S. Animal and Plant Health Inspection Service's (APHIS) Pink Bollworm Rearing Facility in Phoenix, Ariz., is to supply enough competitive, sterile moths to prevent establishment of the pink bollworm in the San Joaquin Valley of California (the only major Southwest cotton-growing area not generally infested). The pink bollworm became a serious cotton pest in California's Imperial Valley in 1966. The first moth detected in the San Joaquin Valley was trapped in 1967. At this time, much of the groundwork in techniques for rearing pink bollworms had been laid for starting up a sterile-insect-release program. This program began on a large scale in 1970.

In the late 1950's and early 1960's, Knipling's (1955) discussion of the feasibility of using the sterile-insect technique and the new emphasis on insect pheromones led to research on pink bollworm nutrition and practical rearing diets (see Vanderzant and Reiser 1956a, 1956b; Vanderzant 1957; Vanderzant et al. 1957; Adkisson et al. 1960a, 1960b; Clark et al. 1961; Ouye 1962; and Ouye and Vanderzant 1964), irradiation (see Bartlett 1978), and mass rearing. Vanderzant et al. (1956), the first study to report on mass rearing the pink bollworm, used diets of immature peas and sprouted beans and of cottonseed and peas. The diet of cottonseed and peas gave better results when insect size was used as an indicator of quality. A food preservative, methylparaben (methyl *p*-hydroxybenzoate) with methyl cellulose, was used to coat the seed and inhibit mold growth for 14 days. These researchers were the first to use antimicrobial substances in the pink bollworm's diet.

Richmond and Ignoffo (1964) were the first to report on attempts to mass rear the pink bollworm on an artificial diet developed by Ouye (1962). Their goal was to produce moths easily and inexpensively for attractant and irradiation tests. They increased the yield per container by using physical barriers between layers of diets. The barriers presumably prevented not only the attraction of neonatal

larvae to light but also movement and competitive interactions in the containers. Richmond and Ignoffo also increased the surface areas by cubing the diet, reducing interactions among neonatal larvae. And they presented data suggesting that the moisture content of diet after drying at 50° C for various periods was responsible for variation in yields. Their data generally showed the highest yield (41%) of mature larvae occurring at 75.7% moisture content and yields decreasing as the water content increased or decreased. An adult-collection system (based on light-trap information of Richmond and Husman 1957) was used to capture 98% of the emerged moths. In a pilot program, 75,225 moths were produced over 45 days when physical barriers of cotton or chipped plastic foam were used between layers of diet in 8-oz glass jars and plastic-coated paper cups and 6- and 9-oz paper cups.

Many of the rearing techniques developed in this early period (see Martin 1966 for a comprehensive review) have been modified or expanded and are now used at the Pink Bollworm Facility. Among these are the use of dark rooms during the first few days of larval incubation, a double thickness of screening to stimulate oviposition, and, most important, adequate sanitation. Martin (1966) foresaw that nearly all the hand procedures for rearing could be completely automated, except the egg-laying culture, and Mangum et al. (1969) reported techniques able to produce 1 million sterile moths per week.

Since the 1968 commitment to a sterile-insect program by the U.S. Department of Agriculture, the California Department of Food and Agriculture, and California cotton growers, techniques and equipment have been continuously changed and refined by the APHIS Methods Development group at Phoenix. For example, use of Honeycomb (Hexcel Corp., Long Beach, Calif.) as a pupation substrate has allowed rapid and safe mass collection of pupae; ceasing to layer with barriers in the diet has saved an immense amount of labor without loss of rearing efficiency; use of caged pupae rather than adults has reduced physical handling and injury to moths and increased oviposition yields; use of larval barriers that restrict larvae to pupation sites has increased the collectable yield; use of a new moth-collection system has increased moth yields and quality; and elimination of pathogens from the rearing facility and the control of fungi and bacteria that multiply in the diet have also increased yields and quality. This paper gives details of these techniques, how we use them at the Pink Bollworm

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Rearing Facility, and what procedures we use to assess and insure quality.

Colony Maintenance and Production

Oviposition and egg incubation

The egg-production units at the Pink Bollworm Rearing Facility are housed in four 3.7-m by 13.8-m housetrailer modified for this purpose. Two units are used for maintenance of three different stock colonies, and two units are used for producing eggs during the production season (mid-April to November 1). The four units hold a total of 2,560 oviposition cages. The genetic integrity of each of the three colonies is maintained as much as possible by strict separation of pupae when the oviposition cages are set up and afterwards by separation in the units. Genetic contamination from another strain (a remote possibility) may result from moths escaping their cages. To prevent this, escaped moths are removed from the units by black-light traps in the trailers and also by daily vacuuming of walls, ceiling, and other surfaces. Moths housed in the production units are a mixture from the three colonies.

Each oviposition cage (fig. 1) is assembled from three major components: the plastic bottom, the fiberboard sidewall, and the steel-mesh top. The white plastic bottom of the cage is a vacuum-formed dish 30.5 cm in diameter with a 5-cm-high sidewall: a central cylinder 6.4 cm in diameter by 3.8 cm long projects from the bottom and attaches to the scale-collection line. A 20-mesh fiberglass screen is cemented to the inside of the cage to prevent moths escaping through the bottom; beneath the screen is a retainer ring that protects the screen when the cage is placed on the scale-collection line. The 11.4-cm-tall fiberboard sidewall is made from the precision cutting of a 3-gal (11.4 liter) size fiberboard sidewall (Sealrite Co., Kansas City, Kan.). The fiberboard fits in a molded groove inside the plastic sidewall. The 20-mesh stainless steel top fits over the sidewall and is sealed with wide tape (6.5 cm) to prevent moth escapes.

Twenty-six cages are set up each day for colony maintenance in each of the three stock colonies, and 100 cages are set up for production. The cages are set over a 90° sanitary tee on a white polyvinyl-chloride pipe (6.4 cm outside diameter, 3.8 cm inside diameter). The pipe not only supports each cage in an upright position but also forms the basis of the sealed scale-collection system. A 2.5-cm² tubular steel rack fastened to the floor supports 80 cages on 10 pipelines. Each of these lines (two each in five tiers) connects to a 5-cm pipeline that, in turn, connects to 15.2-cm pipeline. The largest line connects to three cyclone dust collectors (Ridgeway and Billingsley



Figure 1.—Pink bollworm oviposition cage.

1973) in series on the outside wall of the trailer. Air is drawn through the pipe-and-cyclone system with a 1-horsepower Dayton (AC108), high-pressure, direct-drive blower (Granger, Inc., Skokie, Ill.). The cyclone system removes about 95% of the moth scales and recycles the clean air to the units. Efficiency requires that the cyclones be emptied each day.

In actual use, 49 g of 6- to 7-day-old pupae (about 3,000) are spread evenly over the bottom of the cage. On emergence, usually the 2d day after setup, the moths are supplied a 7.5% sucrose solution made with autoclaved water containing 0.125% methylparaben. Methylparaben is included in the solution to prevent the growth of bacteria, particularly *Pseudomonas aeruginosa* (Schroeter), which is a common contaminant of municipal water supplies and also a pathogen of the adults of several lepidopteran species (F. D. Stewart, unpublished data). One-third of a New Freedom minipad, a feminine sanitary napkin, is soaked in the solution and placed in the hole at the center of the pad, screen, and weight assembly. The minipads are replaced daily with new pads and fresh solutions

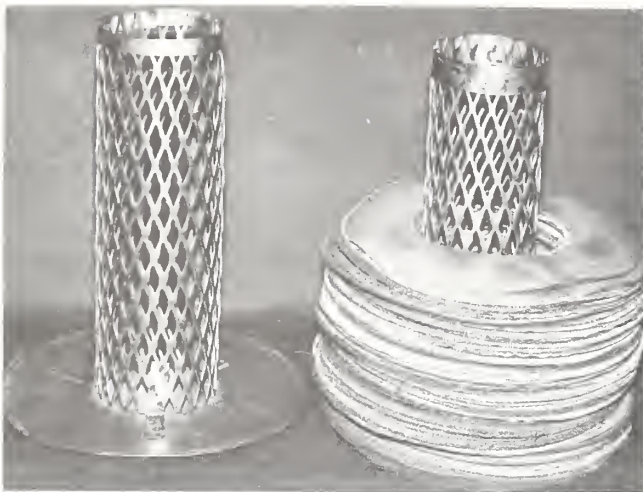


Figure 2.—Spindles used for semiautomated surface disinfection of pink bollworm eggs. Spindle on right holds egg pads and screens.

because they quickly dry out under oviposition conditions of 26.6° C and a minimum of 60% relative humidity.

After the cage has been on the support rack for 4 days, a ring-shaped piece of 18-mesh fiberglass screen is centered on the screen top of the cage. A similarly ring-shaped, flexible weighted pad sheathed with vinyl is put on top of the 18-mesh screen. The weighted pad smooths and brings the paper and screen layers close together. The egg pads with the screen rings are collected on a spindle (fig. 2) each day for 8 days and incubated at 25.5° C for 3 days before disinfection and use. Collection of the screen with the pad prevents damage to the eggs during collection and disinfection.

Surface disinfection of eggs

The egg-disinfection room is a single room maintained under constant temperature; air is filtered by a HEPA (high-efficiency particulate air) filter with positive pressure. The room is equipped with a water-mixing valve to provide water at 28.9° C for solutions and rinse water. A time stamp is used to record the starting and finishing times for each egg-disinfection treatment. On the time-card are recorded solution temperatures and total volume of eggs. Egg treatments are timed so that, preferably, no more than one-half hour elapses from the completion of disinfection to egg implantation in rearing containers. Prolonged submersion in water decreases the hatch rate of eggs, survival of larvae, and yield.

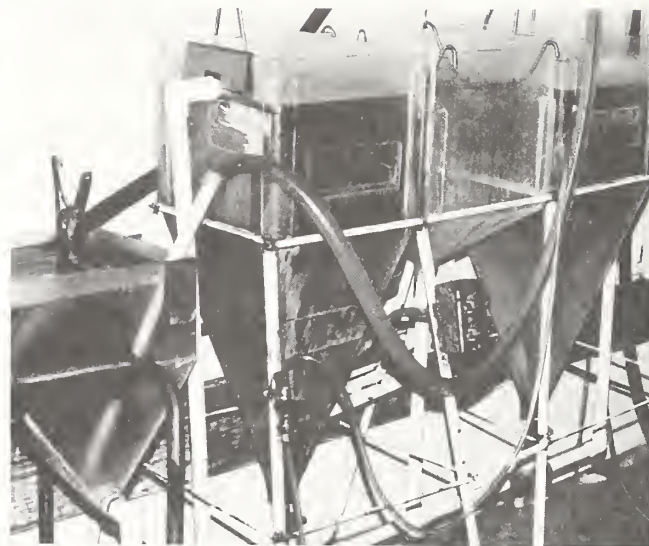


Figure 3.—Semiautomated egg-disinfection system. Long tanks are used for egg disinfection; shorter tanks are used to rinse scales away from the egg pads.

Before the egg spindles are passed into the egg-treatment area, the pads are counted because not more than 140 pads should be on 1 stainless steel spindle. To wet the scales and prevent them from becoming airborne, the pads and spindle are submerged and lightly agitated by hand in 0.26% Tween 80 (polysorbate 80) solution in water at 28.8° C. The eggs are removed from the egg rings and surface-disinfected by a modification of Richmond and Martin's (1966) technique (a 1% bleach solution is used, and the sodium thiosulfate is omitted). In our operation, egg removal and disinfection are facilitated by the use of a motorized semiautomated egg-removal apparatus consisting of three transparent polyvinyl-chloride treatment tanks (38-liter capacity) on a support rack (fig. 3). Agitation is provided to a cradle that supports the submerged egg pads on a spindle connected to a 1/20-horsepower, 30-revolutions-per-minute, eccentric-drive-gear motor (C. H. Billingsley and J. L. Roberson, unpublished data).

In practice, the end tanks are used for the disinfection of eggs and the middle tank for rinses. Normally, a wet egg spindle is put into the middle tank, and a stream of aerated water is directed near the submerged egg rings. The scales stick to the bubbles and are carried to the surface and disposed of via an overflow drain. The egg spindles are then immersed in one of the two outer bleach tanks. Each tank contains 1% bleach and 0.42% Tween 80. One tank of bleach is normally enough for the treatment of three spindles. Eggs begin to loosen and settle to

Table 1.—Ingredients and quantities of 1 liter of artificial pink bollworm diet

Ingredient	Quantity
Agar	g . . . 24.2
Toasted soy flour	g . . . 80.0
Sugar	g . . . 17.6
Wheat germ	g . . . 34.8
Alphacel	g . . . 3.0
Wesson Salts mixture	g . . . 11.6
Methylparaben	g . . . 1.9
Choline chloride (10% solution in water)	ml . . . 11.6
Formaldehyde (10% solution in water)	ml . . . 4.8
Potassium hydroxide (22% solution in water)	ml . . . 5.8
Acetic acid (25% solution in water)	ml . . . 16.4
Calco Oil Red dye	mg . . . 150.0
Corn oil	ml . . . 0.42
Aureomycin (chlortetracycline; 5.5% veterinary grade)	mg . . . 53.7
Fumidil B (fumagillin)	g . . . 0.2
Locust bean gum (optional)	g . . . 1.22–2.44
Potassium pentothenate	mg . . . 90.97
Nicotine acid amide	mg . . . 46.45
Riboflavin	mg . . . 23.23
Folic acid	mg . . . 23.23
Thiamine hydrochloride	mg . . . 11.61
Pyridoxine hydrochloride	mg . . . 11.61
Biotin	mg . . . 0.929
Vitamin B ₁₂	mg . . . 0.0464
Water	q.s. to 1.0 liter

the bottom after 2–3 minutes. After 5 minutes, the eggs are siphoned off into a plastic bucket before being transferred to a 2-liter beaker. Normally, the eggs are exposed to the bleach solution for about 8 minutes. The viability of the eggs begins to decrease drastically after 13 minutes exposure to the bleach solution (J. L. Roberson, unpublished data). Once all the eggs have been collected, they are gently rinsed three times in water (28.8° C) and then disinfected again for 10 minutes in a 9% formaldehyde solution. The final step is to rinse the disinfected eggs four times with autoclaved water. The second disinfection is optional but is used as a prophylactic against any cytoplasmic polyhedrosis virus and *Bacillus thuringensis* (Berliner) surviving the first disinfection treatment (Stewart et al. 1976).

A volumetric estimate of the number of eggs collected is made for each unit. The eggs are poured into a 100-ml graduated cylinder; the volume is noted after they settle for 5 minutes. With 18,000 eggs/ml as a constant, the volume of eggs is converted to the number of eggs.

Diet preparation, storage, and shredding

From the start of the sterile-release program until 1976, the diet used was essentially that of Ouye (1962) except

for the additions of acetic acid, Calco Oil Red N-1700 dye (American Cyanamid Corp., Princeton, N.J.) (Graham and Mangum 1971) dissolved in corn oil, Fumidil B (bicyclohexylammonium fumagillin, Abbott Laboratories, W. Chicago, Ill.), locust bean gum, and potassium sorbate. In 1977, I modified the diet (table 1) by replacing casein as a protein source with toasted Nutrisoy flour (soy flour, Archer Daniels, Midland Co., Decatur, Ill.), and reducing the amount of Alphacel (cellulose powder) and sugar (F. D. Stewart, unpublished data). This diet is similar to a diet developed for *Heliothis virescens* (Fabricius) by Shaver and Raulston (1971). During the production season, three or four batches (558 liters each) of diet are made each day in a 775-liter Groen (Elk Grove Village, Ill.) steam kettle. This amount of diet is used to fill 5,280 rearing containers.

Cooking.—The first step in preparing the diet is to bring the agar to a rolling boil for 5 minutes in about 225 liters of water. Methylparaben, potassium sorbate, and Calco red dye, partly dissolved in corn oil, are added to the steam kettle before boiling to insure the maximum dissolution and dispersion in the final diet. To minimize the preparation time, other diet ingredients are made at the same time into a slurry in a 232-liter kettle with a 0.5-horsepower Lightnen blender, (Mixing Equipment Co., Rochester, N.Y.) and a 0.5-horsepower Tri-Clover blender



Figure 4.—1-quart Convocans used for mass-rearing the pink bollworm.

(Ladish Co., Kenosha, Wis.). The Tri-Clover blender greatly reduces the size of the wheat germ particles. Wesson salt mixture (a nutritional salt mix, ICN Pharmaceuticals, Cleveland, Ohio), sugar, Alphacel, and locust bean gum are thoroughly mixed before the addition of soy flour and wheat germ. All ingredients are mixed for 5 minutes.

The slurry is then pumped into the boiling agar. Potassium hydroxide, formaldehyde, and choline chloride solutions are added separately, in that order, and mixed in. Once the temperature is below 65° C, vitamins, Fumidil B, and Aureomycin (chlortetracycline) are added. Acetic acid is added 5 minutes after the potassium hydroxide. The preparation of the diet is completed after an additional 10 minutes of mixing. The diet is then pumped into 76- by 26-cm stacking fiberglass trays at the rate of 10–11 kg per tray.

Storage and drying.—The diet-filled trays are stored for 3 days. Storage for the first 24 hours is in a large fume hood to facilitate the removal of acetic acid and formaldehyde fumes from the facility (both are toxic to employees and pink bollworm larvae). In a similar diet, formaldehyde completely reacted with the diet ingredients or evaporated over 24 hours, leaving the diet nontoxic (David et al. 1972). The diet is then dried an additional 2 days in a 7.8-m-long, 0.86-m-wide, and 1.8-m-high wind tunnel. A nearly laminar flow of HEPA-filtered, fresh outdoor air passes through the tunnel and is expelled from the building, drying the diet. During high humidity, the drying system can also heat the air before it passes through the tunnel. The diet is dried

because Richmond and Ignoffo (1964) reported that doing so from 84% to 75% moisture produced the highest yields. The APHIS diet contains 79% moisture after it has been prepared. About 1%–2% of the total weight in moisture is lost by the time the diet solidifies; an additional 7%–15% of the total weight is lost during the 3-day holding period. Both poor binding characteristics of the agar and insufficient drying can lower the quality of the diet after it is shredded. Either will produce a wet diet that invariably gives lower yields. I think these lower yields are related more to the amount of free surface moisture released by shredding than to the water content of the diet. Free water can trap or drown the neonatal larvae, or disrupt their normal behavior by causing them to leave the diet and wander about the walls of the rearing container instead of mining and feeding.

Shredding.—The diet is shredded with an Artofex TR-201 (Excelsior Industrial Corp., Fairview, N.J.) commercial stainless steel food shredder with a 5-mm-mesh-size basket. The diet is shredded into fiberglass containers 1.4 m by 0.9 m by 0.3 m. These fiberglass containers are raised to a workable height for the filling of the rearing containers with a mobile, custom-built, motor-driven cable hoist.

Containerization, development, and harvesting

Filling of rearing containers and implantation of eggs.—The container currently used for rearing the pink bollworm is a Sealrite (Long Beach, Calif.) 1-qt Convocan (fig. 4). The inside of the solid container lid is laminated with a polypropylene film that prevents excessive evaporation from the cans. Both sides of the sidewall and bottom of the can are lined with polyethylene film. The cans are made in the facility by an automated machine that forms them from stockboard blanks at a maximum of 38/minute. They are carried to the filling-implantation room via a 23-m-long overhead cable conveyor.

Currently, the can-filling procedure is done by hand. Personnel doing the filling and implanting are required to disinfect exposed skin, wear sterile surgical gowns, foot and head covers, surgical gloves, and beard covers if applicable. About 300 g of shredded diet is handplaced into each container. The disinfected eggs are suspended in a 0.1% sterile agar solution (26.6°–28.9° C) used to dilute and maintain distribution of the eggs during implantation (Richmond and Martin 1966). The normal implant rate may vary according to needs, but is usually 2,000–2,600 eggs suspended in a 4 ml of agar per can; the eggs are dispensed into each container with an F-400X filler pump (Cozzoli Machinery Co., Plainfield, N.J.) and a 20-cc stainless steel syringe (autoclaved each day). The eggs are dispensed directly onto the diet while the can is

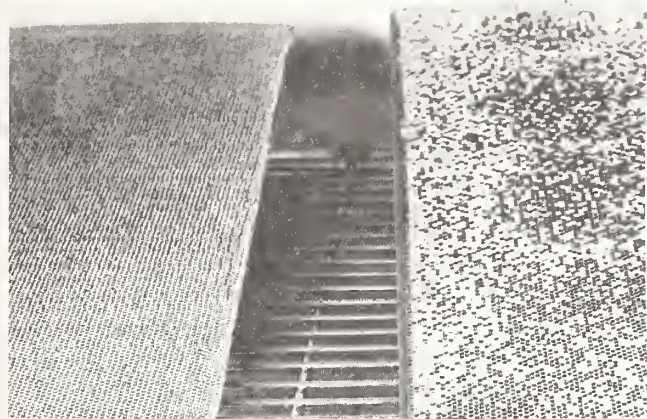


Figure 5.—Honeycomb pupation substrate.

rotated to distribute the eggs in the can. Eggs that are accidentally splashed onto the container sidewalls usually do not hatch, because they dry out; but, even when part of the chorion is removed during disinfection, the egg hatch remains high because of an advanced stage of embryological development (3 days incubation at 26.6° C) at the time of implantation into the moist environment of the shredded diet. Disinfected eggs dry out in 1.5–2 hours when they are exposed to ambient room temperature and humidity. Filled and implanted containers are passed out of the implant area to be stacked in screened-in roller racks and transported by a refrigerated panel truck to incubation buildings.

Although nearly all the filling and implanting procedures had been done by hand, a totally automated system was completed in 1980. The various components—a can filler, implant machine, and can capper—are connected with appropriate conveyor systems.

Incubation of larvae and collection of pupae.—Rearing containers are incubated at 28.9° C for 16 days. The containers are stacked on rearing carts and held in darkrooms for 8 days; each cart is labeled with its unit number, egg-collection and implantation dates, and diet batch. Darkness is required during the early phase of incubation because neonatal larvae wander, are attracted to normal indoor lighting (Mangum and Ridgeway 1968), and could escape. Flashlights or red overhead lights are used to aid personnel working in the darkrooms and to reduce the number of larval escapes. On the eighth day of incubation, the racks are removed from the darkroom and moved to a pupae collection room (cutout room), where they are held for 8 more days. Normal overhead lighting is used when personnel are working in the area, and dim lighting is used otherwise.

In the cutout room, two large, galvanized steel, larval-barrier trays with rippled (Pittsburg seam) overlapping edges (similar to the tray reported by Foster 1978) are placed beneath each stacked tier of 120 cans. An angled, peaked galvanized strip connects the two trays and prevents larvae from falling between them. In each collection tray are centered a 61- by 41-cm piece of corrugated cardboard covered with a 58-cm by 38-cm by 6.4-mm-thick sheet of Honeycomb containing about 20,000 (about 9 cells/cm²) pupation sites 3.2 mm in diameter (fig. 5).

A nondiapausing mature larva behaves the same in a rearing container as it does in a cotton boll; it cuts an exit hole in the carpel or sidewall and drops down to pupate. This behavior by the pink bollworm, unlike that of many other lepidopterans now being reared, allows easy collection of pupae and makes mass rearing the pink bollworm possible. Most larvae enter cells, always pupate with head end up, and usually spin silk caps over the cells. In the Pink Bollworm Rearing Facility, the sheets of Honeycomb with pupae are replaced in the trays on a schedule or by necessity when they are about 60% filled with pupae. A variable loss of pupae and larvae caused by larval competition for pupation sites occurs if the cells exceed 60% occupation. Once the pupae are collected in the Honeycombs, they are not to be stacked tightly together, because the metabolic heat accumulated will hinder future adult mating and sperm transfer, oviposition, and egg viability (Fye and Poole 1971, Henneberry et al. 1977) and cause premature eclosion. Partly filled Honeycomb is replaced twice during the 8 days; each sheet, with the cardboard backing firmly attached by webbing, is labeled by source and stacked horizontally with a spacer (5- by 10-cm fence wire) between each piece.

Another potentially serious problem can be triggered by crowded conditions in Honeycomb. Spores of the bacterial pathogen *B. thuringiensis* are airborne contaminants present in all areas of larval rearing. At least five varieties of *B. thuringiensis* have been identified from isolates from the Pink Bollworm Rearing Facility (C. C. Beegle, personal communication). At first, a low proportion of larvae that have left the containers die from wounds inflicted by other larvae. These wounded and dead larvae readily support the saprophytic growth of these bacteria. If this high larval density is left unattended in the Honeycomb and becomes chronic, the concentration of airborne spores is greatly increased. In time, nearly every dead larva will contain spores, though the cause of death is directly related to wounds. These airborne spores can enter the cans through the larval exit holes on air currents (F. D. Stewart, unpublished data). Larvae may also wander out of a rearing container, become wounded or physically contact *B. thuringiensis* spores, and introduce them into the same or another con-

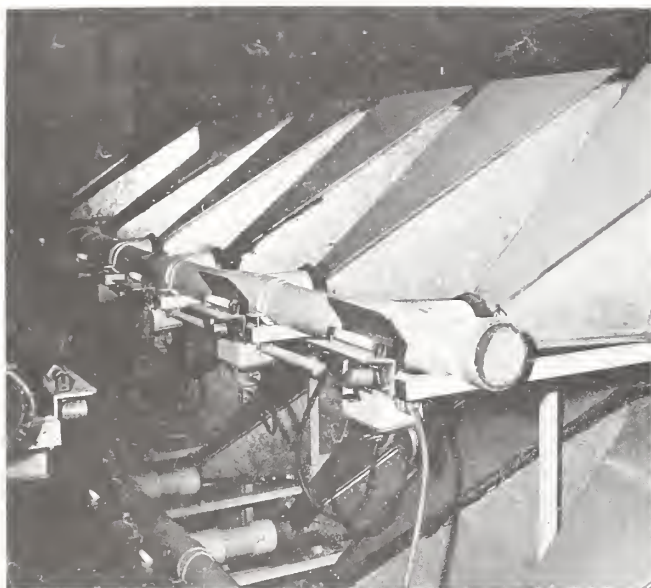


Figure 6.—Moth-collection system showing pupal holding boxes connected to moth-collection lines.

tainer via the exit holes. The problem may be compounded if wounded larvae die in a container after contacting spores via ingestion or wounds. Larvae still in the container are then directly exposed to high concentrations of *B. thuringiensis* spores and crystals from cadavers, and these conditions may start epizootics within containers. Only in these circumstances has *B. thuringiensis* been responsible for a decrease in the yield of pink bollworm at the Pink Bollworm Rearing Facility. Similar problems may arise when other micro-organisms, small beetles, and mites invade or are introduced into the cans through the exit holes.

The pupae used for oviposition are allowed to develop for 7 days (at 28.9° C) and are then collected when the Honeycomb is gently separated from the cardboard sheet. Pupae are allowed to fall not more than 15 cm onto a plastic or cloth surface. Unfreed pupae are dislodged when the Honeycomb is struck gently against a wire frame. Pupa used for the production of sterile moths are incubated for 3–6 days at 28.9° C before they are set up for eclosion at the moth-collection facility.

Moth eclosion and collection.—Tests have shown that the eclosion rate of pink bollworm moths normally exceeds 95%. Our moth-collection system uses a drawn air current to transport moths to collection cyclones housed in walk-in refrigerators (4.4°–5.6°) and averages a collection

rate of 85% (range=80%–95%). A box is used to store the pupae during eclosion. An ultraviolet light attracts the moths from the box directly to the collection airstream. The boxes are large, galvanized 20-gage sheet-metal boxes that taper to a 7.5-cm diameter pipe ending (fig. 6). The pipe end of the box is gasketed and fits into a 7.6-cm right 90° polyvinyl chloride tee. The large end of the box has runners to hold six 44- by 44- by 2.5-cm aluminum trays that allow maximum conduction of heat away from the pupae. A door, gasketed around the edge with neoprene (1.25-cm-wide), fits over the large end of the box to seal it from air and light leaks. A 2.5-cm tubular frame supports the box and permits its pipe end to be easily slid into the 90° tee of the air line. Directly opposite this connection in the pipe wall is a 2.5-cm Plexiglas lens, and immediately behind this is a ultraviolet lamp that radiates through a 2-mm-wide slit directly into the box. The internal flanges of the polyvinyl-chloride pipe have been beveled and polished to prevent injury to the moths. Two lines, each holding eight boxes, are used for the production from one egg-implant day. The daily setup capacity is 3.7 million pupae at the rate of 550 g of pupae per tray.

An air current is drawn through the screened end of the collection pipe with a 0.5-horsepower blower motor. Airspeed (11.9 km/h) is adjusted with a sliding sleeve valve. An airspeed that is too slow will allow the moths to attach to the sidewalls and lenses, obscuring the ultraviolet light and eventually filling the collection tube while further reducing airspeed. Too much airspeed will cause excessive scale removal, injury, or death. In either case, the moth quality and yield are greatly diminished. Prevention of such losses is assured by monitoring the line airspeeds twice daily with a Taylor anemometer (Sybron Corp., Arden, N.C.).

A maximum of 550 g of pupae is evenly spread in each tray and placed in the boxes. The boxes are attached to the collection pipes, all empty tees are plugged, and the pupae are incubated for 7 days at 27.7° C. The boxes must be kept below 32.3° C, a temperature that Heneberry et al. (1977) reported to affect mating in pink bollworm moths. Moths attracted to the ultraviolet light are carried by the air current into a collection cyclone in the coldroom. The top funnel is thickly insulated to reduce condensation, which drowns moths. The bottom funnel is uninsulated to facilitate rapid conduction of heat to inactivated moths which are collected from the cyclone one to four times daily, depending on moth density. A high density of moths in the collectors permits an accumulation of metabolic heat, precludes inactivation, and permits scale loss and moth deaths. Yields are determined gravimetrically for each collector. Emergency boxes are removed after 7 days, and the uncollected insects are killed by freezing.

Quality Control

As in most production endeavors, commercial or biological, product quality at the Pink Bollworm Rearing Facility depends directly or indirectly on quality of the materials and conditions used to produce that product. Diet quality is especially important because it greatly influences insect quality. Quality of environmental conditions is also carefully controlled as is quality of nondietary supplies used.

Diet quality

To successfully mass-rear pink bollworms, all diet ingredients must be of a consistently high quality. To get the best quality ingredients for the least money, practical, pharmaceutical-grade materials are used; and, if the ingredient has a high use rate, a supply contract is negotiated that legally binds the supplier to meet product specifications. When feasible, each lot or batch is sampled at the facility, and the samples are assayed by quality-control personnel or by commercial laboratories before being accepted and used. When an ingredient does not meet product specifications, it is rejected. Rejection of an ingredient has occurred, for example, when vitamins were less than 95% potent, when bleach was less than 5.25% sodium hypochlorite, and when Wesson Salts did not approximate specified composition.

The quality of wheat germ is a special problem because it is relatively unrefined. It is the most variable ingredient in the pink bollworm diet, and annual consumption exceeds 12.7 metric tons. Variation in protein and oil content is caused by weather conditions and soil type where the wheat was grown and by the milling process. Wheat starch and bran in the wheat germ proportionately decrease the concentration of usable nutrients. Instability in the wheat germ caused by prolonged storage produces even more variability in its quality.

Agar is another highly variable ingredient used in relatively large quantity (about 10 metric tons annually). Through experience, we have developed specific requirements for properties of agar for which we conduct our own quality tests before awarding a contract. We require agar of high gel strength, low viscosity at 65° C, and a high water-holding capacity after shredding. Tests routinely conducted include gel strength, viscosity, gelation temperature, and water retention of prepared gels. We test for moisture content of the dry material, particle size, and other standards found in the United States Pharmacopeia. We also make a visual examination for foreign matter. When agar shipments arrive, gel strength is measured for each barrel (45.4 kg); those that do not conform to specified gel strength are rejected.

We cannot afford to buy the diet already mixed by a commercial source because diet quality is so important. Although doing so would seem to be more convenient and economical, we would lose control over a crucial step in diet preparation. If diet problems did occur, we would have much more difficulty tracing the cause if the diet had been bought already mixed and weighed.

Sanitation and traffic flow

Experience has proven that the pink bollworm cannot be successfully mass-reared without control of the insect pathogens and of micro-organisms that make the diet unpalatable. A cytoplasmic polyhedrosis virus was introduced into the facility via the original laboratory culture brought from Brownsville, Tex., in 1969 and continued to hamper insect production until early 1976 when it was finally eradicated. In 1972, production was hampered by severe mold problems, particularly a strain of *Aspergillus niger* (Tieg.) that may have been resistant to methylparaben (a similar resistance problem was detected in 1978). In 1973, the mold problem did not recur, but production was limited by two pathogens, *B. thuringiensis* and a transovarially transmitted *Nosema* sp. Midway through the production season, the *Nosema* sp. was eradicated when Fumidil B was added to the diet. Production reached its low point in 1974 from a widespread epizootic of cytoplasmic polyhedrosis virus.

A milestone was reached during 1974-75 when Stewart et al. (1976) revised techniques for egg-surface disinfection by using 1% sodium hypochlorite solution followed with a second disinfection in 9% formaldehyde solution. These simple changes helped break the infection cycle; so production increased 300% in 1975. During midseason 1975, however, a chronic cytoplasmic virus infection was detected; investigation (F. D. Stewart, unpublished data) revealed that moth scales from oviposition areas were acting as vehicles for polyhedra picked up from adult fecal matter (2% of the scales examined had at least one polyhedral body that could be seen through a microscope). Further observation showed that moth scales (easily dispersed by air currents) were being deposited on rearing containers in the larval incubation rooms and that wandering neonatal larvae (capable of crawling between the container and lid) could pick up contaminated scales and their integument and carry them back into rearing containers. Expansion of these observations showed that mature larvae that commonly exit and reenter rearing containers have a far greater vector potential than do neonatal larvae, because the mature larvae have greater surface area and mobility.

Before 1975, the three parent cultures of pink bollworm were largely self-sufficient because each had its own areas

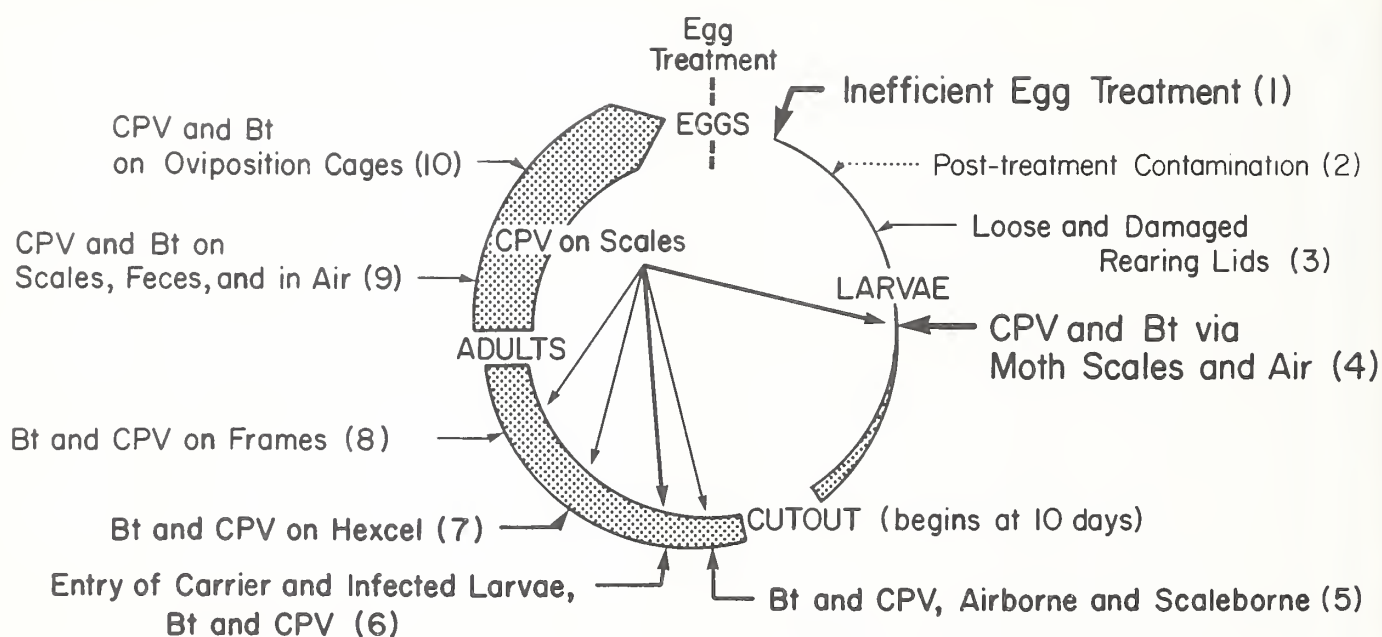


Figure 7.—Sources of contamination at Pink Bollworm Rearing Facility in 1974 and 1975. The increase in width of the circular arrow represents the increase in the contamination load in the facility from different identified sources. (Note: Hexcel is a synonym for Honeycomb.)

of egg-disinfection, container inoculation, larval-incubation, pupation, and oviposition. Sanitation measures were limited to disinfecting work surfaces, floors, walls, and ceilings and to some use of protective garments. The flow of people and supplies from one area to another was given little consideration. Given the key to breaking the cytoplasmic virus cycle late in 1974, the Pink Bollworm Rearing Facility underwent major modification early in 1975. This modification included centralization of all egg disinfection and container inoculation, positive pressurization of diet preparation and egg disinfection, installation of HEPA-filtered air in container-inoculation areas, elimination of people movement from larval-development and oviposition areas to diet-preparation and container-inoculation areas, and extensive use of scrubbing and sterile clothing.

After the discovery that moth scales could carry pathogens, further modification of the facility became imperative. The changes made before the 1976 season were: restrictive routes for people and materials were developed; to eliminate the possibility of contaminated scales settling on disinfected eggs, egg pads were wetted before entering the egg-disinfection room; oviposition areas were semi-isolated; and larval incubation and pupation areas were centralized to take maximum advantage of prevailing winds and to allow logical and effective traffic patterns in the facility. Cleaning of equipment also

became more stringent, especially on those objects likely to be contaminated with scales.

Once the problems were understood and remedial procedures begun, the virus was eradicated within 2 months and has not been detected since February 1976. Because airborne moth scales are much easier to detect than airborne polyhedra, a simple air-sampling technique (24-hour exposure of a gridded filter paper in a 50- by 9-mm petri dish) was used to monitor rates of scale fallout in key areas. Counts of scales on exposed plates were used to track progress and effectiveness of actions taken to eradicate cytoplasmic polyhedrosis virus from the facility. The measures taken to eliminate the cytoplasmic virus have also proven effective against other microbial contaminants. Figure 7 summarizes and illustrates the magnitude of problems associated with cytoplasmic polyhedrosis virus and *B. thuringiensis* at this facility.

Controlling microbial contaminants is not the only key to successful mass rearing of the pink bollworm; levels of airborne contaminants must be continually monitored in all sectors to detect new organisms or increases in quantity. Early detection is the first step in preventing catastrophes. For example, pigmented and unpigmented colonies of *Serratia marcescens* (Bizio), a known pathogen of the pink bollworm (W. L. Belser, personal communication), were detected on air-exposure agar plates.

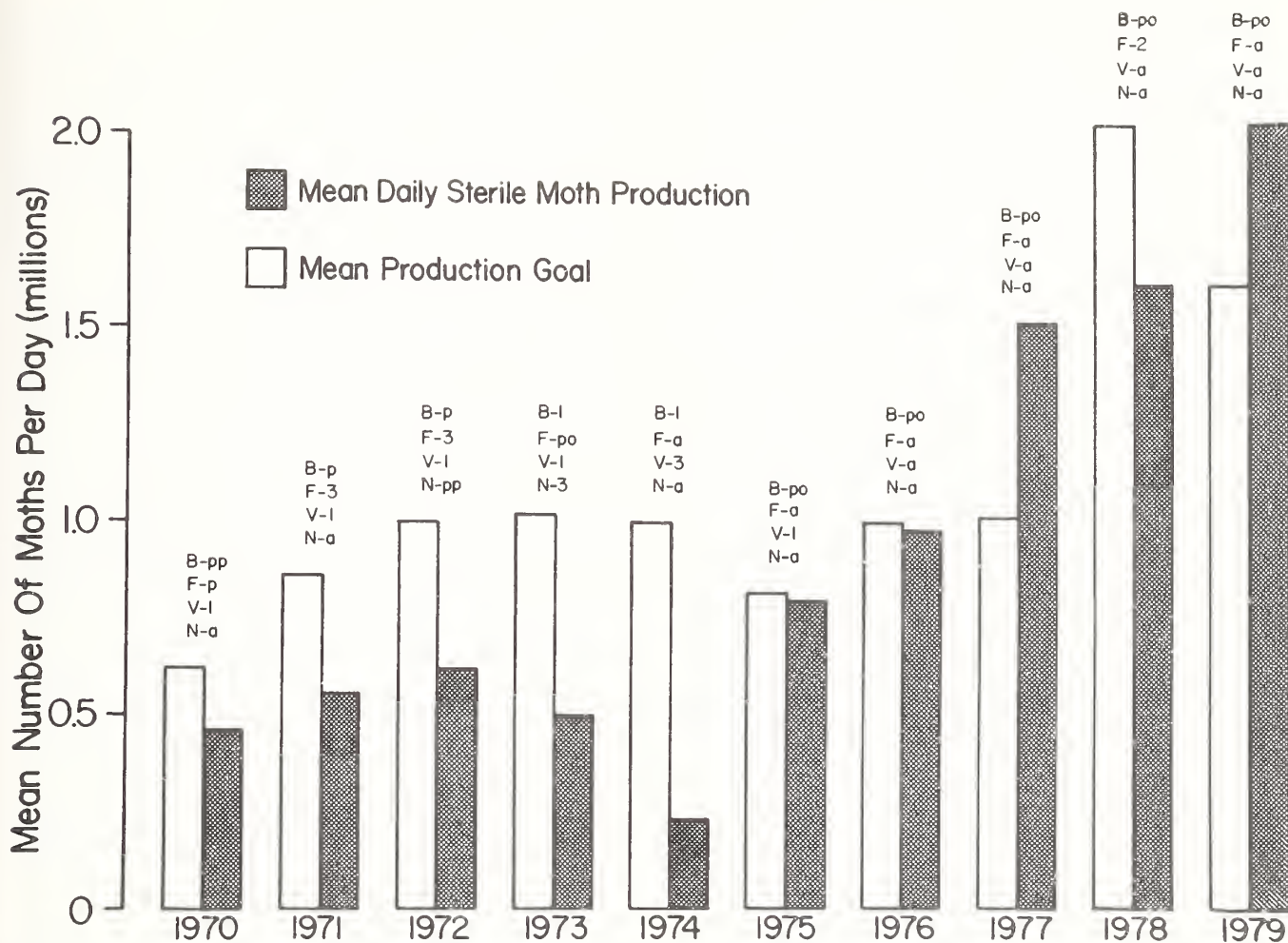


Figure 8.—Summary of the daily production goals for each year from 1970 through 1979 and the influence of associated micro-organisms on the mean daily sterile moth production. Micro-organisms: F=*Aspergillus niger*; B=*Bacillus thuringiensis*; V=cytoplasmic polyhedrosis virus; N=*Nosema* sp. Letters followed by characters indicate presence and effect on production; a=absent; p=present, effect unknown; pp=possibly present, no examination made; po=present, no effect; 1=slight effect; 2=moderate effect; 3=severe effect.

Bacteriological smears were taken from different sites in the contaminated pupation area, the sources were identified, and intensive sanitation measures eradicated the bacterium. Increases in plate counts may be by chance, but they may also indicate serious chronic problems or illicit changes in sanitation procedures or traffic flow.

In general, the measures now used to sanitize the facility include mopping floors and washing walls and ceilings with bleach, quaternary ammonium compounds, phenolic compounds, and stabilized chlorine dioxide solutions.

Chlorine dioxide is advantageous because it is relatively stable and noncorrosive, and it can be rapidly and effectively applied with airless spray guns to almost any surface—particularly walls, ceilings, and supplies entering the facility. All glassware, rinse water, and clothing used in egg disinfection are autoclaved daily. Every failure to meet production quotas at the Pink Bollworm Rearing Facility has been directly or indirectly related to the dominating, harmful effects of micro-organisms (fig. 8), so sanitation is extremely important in insuring that production goals are met.

Insect quality

Thus far, standards used to define overall quality of the insect have been limited by a lack of measurable traits showing how sterile moths will perform in the field. Regardless of what those traits might be, the measurement system must lend itself to routine daily use with several individuals or samples. For example, measurement of flight capability with flight mills (Flint et al. 1975) is not a practical system because of size and fragility of the moths and the nonreproducibility of results (R. T. Staten, unpublished data). Quality traits of sterile pink bollworm moths that have been studied in the field include competitiveness (Van Steenwyck et al. 1979), attractiveness of irradiated females (Flint et al. 1973), and time and duration of pheromone release by irradiated females in the field (P. Lingren, unpublished data). Routine monitoring of these traits would greatly enhance assessment of overall moth quality, but field measurements are expensive, time consuming, and not readily adaptable to routine quality testing. Laboratory tests have only been used to monitor mating and longevity characteristics.

At the APHIS field laboratory in Bakersfield, Calif., irradiated moths are sampled daily just before release. The samples are subjected to various measurements including mating, mating potential, and longevity. Data on mating and mating potential—tests determined by spermatophore counts (Ouye et al. 1965)—are used as tests of handling and shipping procedures and of potential usefulness of moths in the field. Measurement of longevity over 14 days is used to estimate residual or working populations of moths in the field. More importantly, these data are used as signals of production problems (high rates of mortality early in the 14 days have always been disease related) that require investigation. In all cases, interpretation is largely a matter of comparing present performance with past performance.

Other things we might consider as part of the quality-control system include daily monitoring of comprehensive production data (eggs, pupae, and moths produced) and daily measurement of moth size and pupal size. Such data are also used as indicators of potential problems and stimulate thorough investigation.

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Production of Boll Weevils, *Anthonomus grandis grandis*

By J. L. Roberson¹ and J. E. Wright²

Introduction

History and distribution of the boll weevil

The boll weevil, *Anthonomus grandis grandis* Boheman, is a New World pest suspected to have originated in southern Mexico or northern Central America. The first specimen was collected near Veracruz, Mexico, in the 1830's. Its taxonomy was described in 1843 by Boheman from specimens collected in Veracruz. The first report of the boll weevil in the United States was during the fall of 1894 near Brownsville, Tex. By 1898, it had spread across Texas; it was in Louisiana by 1903 and in Oklahoma, Arkansas, and Mississippi by 1907. It completed its distribution across the southeastern Cotton Belt of the United States by 1922. (For a detailed history and taxonomy of the boll weevil, see Burke 1968.) The boll weevil develops on cultivated and wild cotton of the genus *Gossypium* and other closely related genera. (For more information on host plants of the boll weevil, see Cross et al. 1975.)

Life cycle of the boll weevil in the field

The boll weevil is a holometabolous insect with an egg, larval, pupal, and adult stage. The life cycle may be completed in 18–21 days at temperatures of about 30° C and 60% relative humidity, so five or more generations may occur each year. The adult is 6–7 mm long, weighs about 15 mg, and ranges in color from tan to dark gray or brown. The proboscis is about half the length of the body, and the adult has a spur on the inner surface of each front femur.

With its proboscis, the adult punctures squares (flower-buds) or bolls for feeding and oviposition. The females lay eggs in feeding punctures and then seal the holes with a gluey substance. These eggs hatch in 3–5 days; hatching is followed by a 7–12 day feeding period that causes the squares or bolls to abscise. Larval feeding is completed in the fruit on the ground. The pupal stage lasts 3–5 days

and is followed by adult emergence. Female weevils begin to lay eggs 3–7 days after feeding and mating. This life cycle occurs repeatedly until cotton is no longer available. Then, adult weevils overwinter in diapause in surface woods trash, along ditch banks, in cottonfields, and in trash and litter around gins and farm buildings. Diapause generally ends in the spring about the time squares are being formed on the cotton plant.

Laboratory Rearing of the Boll Weevil

Rearing procedures for the boll weevil at the U.S. Animal and Plant Health Inspection Service's Robert T. Gast Boll Weevil Rearing Facility are a compromise between the insect's basic necessities and the artificial conditions required to produce the numbers and quality of insects needed for research and proposed field testing of sterilized weevils. Insect-control plans using sterile-insect techniques demand massive numbers of high-quality insects to be produced and delivered on specific use dates. Since mass-rearing procedures were first reported (Gast and Davich 1966), research has been directed to development and incorporation of mechanized operations where possible. The aim is to reduce risks of microbial contaminants in sensitive work areas and increase production capacities. Most recent advancements include incorporation of quality-control techniques to identify problems and evaluate production standards.

Colony selection

The colony presently being mass-reared at the R. T. Gast Rearing Facility originated from an ebony strain selected by Bartlett (1967); this character is now used as a marker to distinguish the laboratory weevils from native weevils. The ebony characteristic is semidominant and requires selection and backcrossing from the colony for its maintenance. In 1975, native specimens were collected in North Carolina and crossed with laboratory ebony; the progeny were selected for ebony color. In October 1978, ebony adults were selected from pupal cells; these adults were colonized, and the following generation was inspected and nonebony weevils discarded. The selected colony was isolated and increased to replace the existing colony. Adults reared for release in the 1979 North Carolina boll weevil eradication trial were inspected daily; they exhibited 98% ebony characteristic. Calco red dye was added to the larval diet (Lindig et al. 1980) to provide an additional marker.

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Table 1.—Contents of a premix batch of dry ingredients for adult and larval diets of the boll weevil

Ingredient	Amount (g)	
	Adult diet	Larval diet
Cottonseed meal, sifted	28,500	31,800
Agar	8,400	8,280
Sugar	6,850	7,800
Promine D	10,800	12,280
Corncob grits, 60 mesh	6,000	
Cholesterol	300	360
Wesson salt mix	1,125	1,260
Vitamin mix ¹	1,170	1,260
Potassium sorbate	585	400
Methylparaben (methyl <i>p</i> -hydroxybenzoate).	375	400
Ascorbic acid	270	290
Methenamine	60	
Cottonseed meats	10,800	10,724

¹See table 2.

Laboratory rearing procedures

Preparation of diet premix.—Diet ingredients are received in bulk. As each shipment of ingredients is received, it is identified either by the date received or by the industrial-batch-lot number. As each premix batch of dry ingredients (tables 1 and 2) is prepared, it is identified numerically; the industrial- or storage-lot number of each ingredient used is recorded for reference. This record makes it possible to find out when new ingredients were introduced into the production line and provides valuable information if diet quality is questioned. Two records are made for each premix batch; one remains on file in the weighing area, and the other is attached to the diet container. The work operations for diet preparation are: All ingredients are weighed with top loading balances. Cottonseed meal is sifted through a 32-mesh sieve screen with a 2.5-horsepower automatic feeder/sifter/shaker (Griffin and Lindig 1974b). All ingredients except agar, Promine D (soy protein), and corncob grits are weighed and loaded into a heavy-duty mixer. These ingredients are mixed about 20 minutes to obtain a uniform distribution of cottonseed meats. The bulk mix is transferred by conveyor to a hammer mill (Griffin 1979) where it is milled and returned to the blender. The remaining diet ingredients are weighed and added to the blender for a 30-minute final mixing. When the blending operation is completed, the mixture is resifted, packaged, labeled numerically, and transferred to the diet-preparation area.

Operation of the equipment to prepare the blended diet mixture causes dust problems in the rearing area. The

Table 2.—Contents of vitamin mix in a premix batch of dry ingredients for boll weevil diet¹

Ingredient	Amount (g)
Niacinamide	20
Calcium pantothenate	20
Riboflavin	10
Thiamine hydrochloride	5
Pyridoxine hydrochloride	5
Folic acid	5
Inositol	40
Sugar	6,160

¹Ingredients weighed on balances and then ball-milled for 1 hour to give a homogenous blend.

dust contains diet ingredients that harbor bacterial and fungal growth. So the work area is well-ventilated to minimize dust inhalation and development of potentially explosive conditions. Also, the work area is maintained in a separate building to minimize threat of microbial contamination to the rearing colony.

Diet preparation.—Both adult and larval diet formulations are mixed with water and flash-sterilized in one of the three No Bac Unitherm IV (Cherry-Burrell Corp., Cedar Rapids, Iowa) processing systems (Griffin and Lindig 1974b). The flash-sterilizing units are interchangeable and serve to back each other up in case of mechanical malfunction. Diet is prepared at the rate of 151 ± 19 liters per hour. It is sterile as it enters the stainless steel transfer lines (0.7 i.d. by 1.0 cm o.d.) going to the desired area. Three forms of diet are prepared in the room: pellets for adult oviposition, slabs containing diflubenzuron³ (Wright, McCoy, et al. 1980) for feed-out of weevils to be irradiated, and larval diet for rearing trays. Before larval diet preparation, the tubing used to transfer sterile diet is flushed free of a disinfectant solution (1.0% Mikro-Quat,⁴ Economics Laboratory, Atlanta, Ga.) and treated with Oxine⁵ (Bio-Cide Chemical Co., Norman, Okla.) at 500 p/m (parts per million). The Oxine is introduced and maintained in the lines for 15–20 minutes. After use, the equipment and transfer tubing are treated with a strong, oxidizing soap to rid the system of adhering organic matter, then neutralized with an acid detergent. After standard clean-up procedures, a germicidal agent (1.0% Micro-Quat) is pumped into the lines and held until the next use.

³N-[[[4-Chlorophenyl]amino]carbonyl]-2,6-difluorobenzamide.

⁴Active ingredients: Alkyl (50% C₁₄, 40% C₁₂, 10% C₁₀) dimethyl benzyl ammonium chlorides, 9%; trisodium

⁵Active ingredient: chlorine dioxide, 2%.

Pellet preparation.—A batch of diet is mixed, sterilized, and pumped to the pellet-forming unit. The pellet-forming unit is constructed with 12 stainless steel water-jacket lines. The diet is pumped into each of the lines at 12-second intervals. Cold water flows continually around each line, causing the diet to gel; introduction of liquid diet into the line with gelled diet forces the gelled diet out, in rod form, and into a rotating wire wheel that cuts the diet into pellets (0.8 cm in diameter by 1 ± 0.5 cm long). The pellets fall into a vat containing a mixture of 40% beeswax and 60% paraffin maintained at 66° C. The pellets submerge and are completely coated with the wax mixture. As they settle in the wax mixture, the pellets are collected on an inclined endless wire belt that transfers them from the vat to a catch container. They are then poured from the catch container into pans (24.1 cm wide by 13.8 cm deep by 40.9 cm long) for holding and storage in a cool room; or they are transferred to the oviposition room (Griffin and Lindig 1974a).

Slab preparation.—The adult-diet formulation containing 100 p/m diflubenzuron (25% wettable powder) is processed by the flash-sterilizing system. Processed diet is cooled to about 71.1° C and transferred through stainless steel tubing to a Mateer-Burt diet-filling unit (Mateer-Burt Co., Wayne, Pa.). The unit is calibrated to dispense 285 ml of liquid diet into serving trays (34.3 cm wide by 1.9 cm deep by 45.7 cm long). The trays are positioned in a chain drive that moves them through a chill tunnel (53.3 cm wide by 172.7 cm long) to gel the exposed diet surface. The machine fills and delivers six gelled trays per minute for positioning in mobile storage racks (94-tray capacity). The racks with trays are transferred to the adult feed-out area for use. The trays are washed and autoclaved before reuse.

Preparation of larval trays.—Sanitation is vital to successful preparation of the larval trays. So it is done in an isolated room with maximum security for sanitation; the room is restricted to assigned personnel who must wear clean cover clothing when entering and working in it. All air-conditioning outlets in the room have absolute HEPA (high-efficiency particulate air) filters and operate 24 hours a day. Microbial levels are routinely monitored. About 15–20 exposure plates prepared with trypticase soy agar (TSA) growth medium are put in specified places in the room for 20 minutes and exposed to atmospheric microbial settlement while trays are being prepared. Touch plates prepared with the TSA growth medium are used to press against surface areas of materials and equipment to obtain samples showing levels of microbial contamination. Samples of tray components such as diet, eggs, and sand-corn-cob mixture are taken with each new batch lot that is introduced into the production line (Sikorowski 1975).

Trays for larval development are made from a stock roll of polystyrene that is 15 cm wide by 18 mil thick. The polystyrene sheeting is heated to make it pliable. A vacuum is introduced, and the polystyrene is pulled within a mold that forms cavities in the sheeting. The sheeting with cavities serves as a larval rearing tray. It is secured and moved by clamps on the Anderson Form-Fill-Seal Machine (Model 655-B, Anderson Bros. Manufacturing Co., Rockford, Ill.) in a synchronized fashion that mechanically completes all tray-preparation tasks. (See Harrell et al. 1977 for the original concept and design of tray-forming equipment in boll weevil rearing. Accessory equipment to dispense and chill the diet and dispense the eggs and sand-corn-cob mixture was improved by Griffin et al. 1979.)

The larval diet formulation differs from the adult formulation in ratio of ingredients; however, processing procedures with the flash sterilizer are the same. The sterile diet is delivered to a dispenser that is synchronized with the Anderson Form-Fill-Seal machine; and, as the sheeting passes below the dispenser, each cavity is filled with 185 ml of liquid diet adjusted to $40^\circ \pm 10^\circ$ C. This temperature permits the diet to flow evenly in the cavity but gel quickly with a minimum of cooling. The cavities filled with diet enter a 1.5-m-long cooling tunnel maintained at 1° C, a procedure that gels the diet surface. The clamp track moves the cavities from the tunnel and positions them beneath a pump and sprayer that deliver 4 ml of eggs (2,100 eggs) in a furcellaran solution (0.5%) to the diet surface. The furcellaran solution is used to suspend the eggs, thus enabling uniform distribution with spraying. The clamp track advances the cavities with diet and eggs below a hopper that dispenses a sterile sand-corn-cob (70 : 30) mixture containing antibiotics (Sikorowski et al. 1980) over the diet surface to absorb moisture and force hatching larvae to feed on the diet. The clamp track then moves the cavities into position so that a Tyvek (Standard Packaging Corp., Atlanta, Ga.) cover can be heat-sealed over the surface. Tyvek is a porous polyethylene material that allows air and moisture exchange in the cavities. The clamp track moves the sealed cavities to a shearing station that cuts the sheeting into trays, each with two cavities. The trays are agitated by hand with a circular motion to spread the sand-corn-cob mixture over the diet surface then manually positioned on metal tracks in rackveyors (Griffin 1979) for transfer to the larval holding area. The rackveyor is designed for maximum space efficiency, mobility, and tray suspension for good air movement around trays.

A record sheet to aid in monitoring of production processes is attached to the rackveyor and remains as a reference for quality control. Data corded on the sheet identify the trays when different lot numbers of tray components or abnormal procedures are introduced or occur in

the production line. Data are normally recorded on rearing trays by tagging them when changes occur in the diet-preparation batch number, the polystyrene roll, the Tyvek roll, the bottles containing egg suspension, etc. All trays on the rackveyor are inspected for loose Tyvek covers; if any are found, they are sealed with tape before transfer of the rackveyor into the larval holding room.

Larval development.—The larval-development room is maintained at about 31° C, 55%±5% relative humidity, and 24 hours of light. Egg hatch occurs within 3 days after eggs are put in trays; larvae feed vigorously. Pre-pupae forms appear in isolated cells by the 9th day, and adults can be observed in the trays by the 13th day. The larval holding room is kept clean with an absolute air-filtering system, and all personnel are required to wear clean clothing when in the area. Temperature and humidity are monitored to insure proper drying of the diet for normal insect development. If abnormal conditions arise and adults emerge earlier than usual, the rackveyors are removed from the area to prevent contamination in the cleanroom. On the 13th day after eggs are put on the tray, the rackveyors are transferred to the adult emergence area.

Adult emergence and collection.—The rackveyor containing trays of emerging adults is stationed in the workroom. A small hole is melted in the exposed end of each tray with a heating element, and the rackveyor is then transferred to a darkened emergence chamber (2.4 by 2.0 by 2.2 m). Twenty-eight 3.78-liter plastic jars are positioned over openings (5 cm in diameter) in the outside of the chamber wall. Light entering the chamber through the jar portals attracts emerging adults, which become trapped in the jars. Adults are harvested from the collection jars daily, more often if necessary to prevent damage to collected adults. Adults emerging on days 1 and 2 are transferred to laying cages and used as maintenance stock. Adults emerging on days 3, 4, and 5 are bagged in feeding packets and fed for 5 days before irradiation. The rackveyor with spent trays is transferred to a heating chamber and treated for 24 hours at 52° C to kill weevils remaining in the trays. The trays are bulk-packaged in two-ply paper waste-disposal bags, sewn closed, and discarded.

Adult feed-out for colony maintenance.—The adult weevils for egg production are maintained for 14 days at 28° C, 50%±5% relative humidity, and a photoperiod with 20 hours of darkness, 4 hours of light. These adults are held in stainless steel cages (45.7 by 96.5 by 5 cm), described by Griffin et al. (1979). These cages are constructed with removable tops and 12- by 12-mesh stainless steel cloth bottoms. About 9,000 weevils are placed in each cage (estimated by weight). The cage contains two baskets (41.2 by 41.2 by 1.2 cm) to hold diet pellets that serve both as food and as oviposition sites. After the first 2 days, the

eggs are mechanically extracted from the pellets (Griffin and Lindig 1977), cleaned, and surface-sterilized (Sikorowski 1977). They are then mixed with a sterile furcellaran solution (0.5%) for suspending and dispensing in rearing trays.

Adult feed-out for irradiation.—Adult weevils collected for irradiation are placed in 20.5- by 40.6-cm (mosquito netting) bags (6,000/bag; O. T. Malone, unpublished data) and maintained for 5 days on diet containing antibiotics and 100 p/m diflubenzuron (Wright, Roberson, and Dawson 1980). They are transferred to fresh diet daily. Environmental conditions of the feeding rooms are maintained at 28.3°±1° C, 50%±5% relative humidity, and 20 hours of darkness. On the morning of the sixth day, the bags are transferred to a walk-in refrigerator, and the weevils are chilled until immobilized (30 minutes at 2.7°±1° C); the dead or very small adults are separated by processing in a seed separator. About 130,000 adults are placed in a Plexiglas canister, (28.2 cm in diameter by 10.2 cm deep; J. G. Griffin, unpublished data) and transferred to the irradiation area for processing. The weevils in canisters are irradiated with 10 krad from a cesium source and packaged in cool boxes for shipment to release sites.

Maintaining production yields

To insure that the required quantity of boll weevils is produced, production records are kept in each rearing section. These provide a quick review of each production area so that potential rearing problems can be identified. Effective control of production is achieved by recording and monitoring production procedures as follows:

1. When diet ingredients are weighed, the industrial batch number is recorded and maintained with each diet batch.
2. Ingredient and operational variables are recorded during diet preparation.
3. Records of tray preparation identify trays that may be affected by changes or adjustments that have occurred.
4. The eggs-per-female production rate is recorded daily.
5. Adult emergence for each egg-implantation date is monitored and is compared with estimates of adult production based on numbers of eggs implanted, hatch rates, and larvae per tray.
6. Egg hatch for each implantation date is monitored as an indicator of viability.
7. Microbial contamination in cleanrooms is continually monitored to maintain sanitation standards.

The collection and use of these data provide information essential for management to meet production goals.

Quality Control

Quality-control data are needed to certify that all rearing and sterilization processes are functioning properly so that the insects produced meet established standards. Data obtained from sampling boll weevils irradiated for release are as follows:

1. Bacterial gut-load level (Sikorowski 1977). Twenty adult boll weevil specimens are collected from each egg-implantation date. Weevils are analyzed and classified into groups I-IV according to their bacterial gut-load level.
2. Sperm transfer. Twenty-five paired matings of irradiated and nonirradiated adults are made from each day's production.
3. Pheromone production. One hundred males are analyzed for pheromone production (McKibben et al. 1976).
4. Egg viability of irradiated adults. A gross sample, about 9,000 irradiated mixed-sex adults, are collected from irradiation canisters daily and maintained in laying cages for 5 days. Eggs are collected daily and incubated to determine viability.

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Mass Production of Screwworm Flies, *Cochliomyia hominivorax*

By Harold E. Brown¹

Introduction

The mass production and release of irradiated sterile screwworm flies, *Cochliomyia hominivorax* (Coquerel), is the first and most outstanding program to control an insect pest by release of the sterilized insects. The development of rearing techniques on artificial media (Melvin and Bushland 1936, 1940), the initial concept of the sterile-male technique by Knippling in 1938 (1955, 1959), and the determination that viable sterile males could be produced after irradiation of the pupae by X-rays (Bushland and Hopkins 1951) and gamma rays (Bushland and Hopkins 1953) provided the technology that led to the completion of successful screwworm eradication programs. Sterile-male release has eradicated screwworms from the southeastern (Knippling 1960) and southwestern (Bushland 1975) United States, Puerto Rico (Williams et al. 1977), and twice from the island of Curacao, Netherlands Antilles, (Baumhover et al. 1955, Coppedge et al. 1978). Billions of screwworm flies have been artificially reared, irradiated, and released during these control programs.

Presently, the U.S. Department of Agriculture's Southwest Screwworm Eradication Program at Mission, Tex.,² and the Mexican-American Program for the Eradication of the Screwworm at the rearing plant located in Tuxtla Gutiérrez, Chiapas, Mexico, rear a combined total of about 500 million screwworm flies each week. This report summarizes techniques for strain development, selection, production, and quality control used by these programs for the production of high-quality screwworm flies.

Colony Selection

Selection of flies used in the colony for mass rearing is divided into two operations: the development and selection of candidate strains and the introduction of the strain of flies to be used in the rearing colony.

Strain development and selection

Strains of screwworm flies to be used in the mass-rearing facility are usually generated from egg masses collected from areas that will be treated with the sterile flies (Crystal and Ramirez 1975, Crystal and Whitten 1976). Egg masses are collected from naturally or intentionally wounded animals and transported to the laboratory for incubation, larval rearing, and strain expansion. When egg masses are collected in remote areas, the larvae are often reared in temporary laboratories and the pupae transported to the permanent laboratory for further development. More recently, strains have been developed for release back into the specific geographic locations they are derived from. It is hoped that matching locations in this way will take advantage of selective mating if, in fact, native females are more likely to mate with males derived from the same geographic area.

In the southeastern eradication program (Baumhover et al. 1966), efforts were made to collect egg masses from scattered areas to get a wide genetic base. These strains were selected for sexual vigor, longevity, and resistance to starvation to get aggressive, artificially reared flies. The early strain (Baumhover et al. 1966), labeled "the Florida strain," was successful in eradicating screwworms from the southeastern part of the United States. The Florida strain was also used for mass production when the Southwest Screwworm Eradication Program was begun in 1962 since time and resources for field-testing candidate strains were inadequate. (See Bushland 1975 for a discussion of strain development and changes in strains for mass production through 1974.)

How many and what kinds of laboratory and field evaluations can be used in selecting strains for mass rearing are limited. But all candidate strains are tested in the laboratory for larval weights, pupation, emergence, mating, fertility, oviposition percentages, longevity, and response to heat and starvation stresses (Crystal and Ramirez 1975, and Crystal and Whitten 1976). Biochemical, genetic, and physical measurements have also been used

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²Since I wrote this paper, the program at Mission, Tex., has been closed down. The eradication program at Mission was under the auspices of the U.S. Department of Agriculture's Animal and Plant Health Inspection Service, which shared a location with a screwworm research program of the U.S. Department of Agriculture's Agricultural Research Service.

sometimes to compare strains. Isoenzyme (Whitten 1980) and electroretinographic (Goodenough et al. 1977, 1978) techniques have been used and added to the series of evaluations to determine whether a strain is suitable for mass production and release by the eradication program. Also, field evaluations similar to that reported by Ahrens et al. (1976) have helped assess candidate strains for mass production. These evaluations use trap-back studies of released flies from the various strains and provide information about the migration, dispersal, and survival of the flies.

Colony introduction

At Mission, once a strain has been selected, it is expanded in the U.S. Agricultural Research Service's screwworm research facilities; then pupae are transferred to the methods development section of the U.S. Animal and Plant Health Inspection Service's Mission facility at a rate of about 4 liters/day for further expansion. Currently, strain expansion takes place in the methods development section where enough flies can be reared for production and for field tests before strain introduction. The expansion is timed to provide pupae for 21 days, a period that covers a one-generation cycle of the fly. Production is reduced during strain changes since, to prevent contamination, all rearing areas (fly colony, larval starting rooms, rearing floors, and pupation and pupal holding rooms) are cleared of all the previous strain before introduction of the new strain. During these strain changes, problems often occur in establishing proper oviposition, egg-incubation, and starting and rearing schedules. These production bottlenecks must be overcome, and the new strain must adapt to the laboratory environment.

Colony Maintenance and Oviposition

About 5% of the flies produced are used for the brood colony and for egg production. The brood colony is selected from the normal system for pupae handling at about the midpoint of each work shift. Pupae are collected just after pupation and are placed in screen-bottomed trays (46 by 66 by 5 cm), 2.5 liters/tray. Trays of pupae are held in a constant temperature (24°–25° C) and relative humidity (50%–60%) in a separate room for 6 days and then placed in the colony cages. These metal cages (109 cm wide by 152 cm long by 180 cm high) are stocked with 2 trays of pupae (about 50,000) each. Emergence occurs in 24–48 hours. Cages are equipped with five rows of paper curtains hanging from rods running lengthwise in the cages. These provide resting areas for the flies. A bun tray (46 by 66 by 2.5 cm) containing 10.8 kg of adult diet (50% nutria meat and 50% honey covered with a thin layer of a 50 : 50 mixture of cot-

tonseed hulls and rice hulls) is provided for each cage. The Mexican program uses horsemeat, instead of nutria, in the same proportions. The cages are also stocked with 10 containers of honey (500-ml-jar chick feeders) and 10 containers (1 liter each) of water. The cages are placed in the colony room (26°–27° C, 50%–60% relative humidity, 13 hours light, 11 hours dark) and held 7½–7¾ days before oviposition. Each shift, the cages are moved forward one step in a sequence that moves the cages around the colony room to the oviposition room. At the Mission mass-production facility, 7 cages are filled with eggs (egged) each shift (21 shifts/week) for production of 200 million flies/week. More cages are used at Tuxtla Gutiérrez per shift for production of 300 million to 350 million flies/week.

Mating occurs during the holding process; it begins about 1 day after emergence and is completed in about 5 days. Oviposition occurs about 16 hours after the flies have reached ovarian maturity (stage 10, according to Adams and Reinecke 1979), which is about 7½–7¾ days after emergence. The time required to reach maturity varies slightly with each strain and usually shortens in the laboratory. It would be useful in the mass-production facility to egg the cages at the proper time after reaching stage 10. But, under mass-production schedules, each function must be performed with each shift; so, a group of flies must be egged when eggs are needed whether or not the flies are at the best time for oviposition.

For oviposition, the cages are equipped with oviposition vats and boards and moved into a dark room (26°–27° C). The oviposition vat, which is preheated, contains a foam-rubber mat, eggging attractant, eggging boards, and a lighting system. The aluminum vats (160 by 30 by 2.5 cm) are thermostatically controlled at 37°–38° C. The foam-rubber mat is cut to fit and is saturated with the eggging attractant (6 liters/vat), which is a 50 : 50 mixture of used liquid media and water with about 2 liters of citrated whole blood added. The eggging boards are a box-like arrangement of four 1-by-4 boards (152 cm long) running lengthwise and held together by a 1-by-4 board (20 cm long) across each end. These boards are placed edgewise on top of the rubber mat. The lighting arrangement (a hood reflector and three 7½-watt light bulbs) is placed above the eggging boards. The lights and attractant cause the flies to migrate to the boards, where oviposition occurs. Flies are allowed to lay eggs for 4 hours. Then the cages and contents are placed into a cold-room (3°–5° C) to inactivate the flies. Eggging vats are removed, and the flies are destroyed by freezing and burning. Eggs are removed from the eggging boards with a spatula and prepared for incubation. Eggs are weighed in 7-g batches (6 g at Tuxtla Gutiérrez because the rearing vat is smaller), placed in moistened petri dishes, and incubated at 25° C for 12 hours until hatched. After

hatch, the larvae are transferred to the starting room where they are placed in starting pans for rearing.

Production

Mass production of screwworm flies has been described by Graham and Dudley (1959), Smith (1960), and Baumhover et al. (1966). These descriptions were based on technology used during the southeastern eradication program. In the current program, many of the techniques are similar, but others have been changed because it is larger and more complex than the earlier program. One of these changes is the present use of liquid nutrient medium for feeding the larvae. The liquid medium is cheaper and more readily available than various types of flesh. Both the southwestern and Mexican-American programs use the liquid nutrient medium for larval rearing; the only meat used in the rearing facilities is that used in adult fly diets. The use of liquid medium has been facilitated by the research of Gingrich (1964) and Gingrich et al. (1971) and the modifications of the medium resulting from the research done by H. E. Brown and J. W. Snow (1978, 1979, and unpublished data). The development of this medium simplified logistic problems associated with acquiring and storing medium components, medium preparation and handling, feeding schedules and procedures, and waste disposal.

Starting larvae

Eggs collected from oviposition cages are incubated in petri dishes. After eclosion, the larvae are distributed evenly in fiberglass pans (64 by 45.7 by 7.6 cm) rimmed with dry whole egg containing about 2.5 cm of liquid starting medium suspended on either cellulose acetate fibers or cotton linters for support. The liquid starting medium is suspended at a rate of 30 liters of medium to 0.92 kg of shredded cellulose acetate or 1.82 kg of cotton linters. The starting medium used at Mission consists of 6% dried whole blood, 3% dried whole egg, 3% calf-milk supplement, 87.8% water, and 0.2% Formalin (formaldehyde) as a preservative. Starting medium used at Tuxtla Gutiérrez is the same as that used for general rearing and consists of 8% dried whole blood, 3% dried whole egg, 3% dried nonfat milk, 0.2% Formalin, and 85.8% water.

The starting pans are held in a room at 38° C and about 90% relative humidity for 40–44 hours. The larvae are provided supplemental feed as needed. After the 40–44 hours, larval weights range from 5.5 to 7.5 mg, and larvae are ready for transfer to the mass-rearing floor. The starting room provides a less hostile environment than the mass-rearing floor, allows good early growth, and provides for the production of larger larvae. Its use also saves space on the rearing floor.

At Mission, the mass-rearing vats are covered with a 2.5-cm-thick cellulose acetate mat (0.92 kg) and saturated with liquid nutrient medium (about 30 liters). The medium (85.8% water, 8% dried whole blood, 3% dried whole eggs, 3% dried nonfat milk or 3% calf-milk replacer, and 0.2% Formalin) is prepared in a medium-mixing room outside the rearing plant and piped to the rearing floor. The medium is added to the vats through hoses with radiator-filler nozzles. The hoses lead from the overhead medium-supply system. Rearing vats are preheated to about 37° C. The contents of the starting pans (one pan per rearing vat) are distributed evenly on the surface, and the larvae are allowed to work into the medium. Vat temperature is maintained near 38° C. During the first 16–20 hours, supplemental nutrient liquid is provided as needed to prevent drying and excess larval crawling and to provide added nutrients. Twenty hours after larvae are put in the vats (corresponding to a larval age of 60–64 hours) and at subsequent 4-hour intervals, the spent medium is removed from the vats with a specially designed vacuum system.

During the rearing process, larvae that leave the rearing vats prematurely are swept up and returned for further feeding. Those larvae that leave 44 hours or more after being introduced into the vats are collected for pupation. Larvae are collected by a flowing-water system that transports them to a sump where they are pumped through a separator that shakes water and larvae apart. Larval feeding is ended after the larvae have been on the rearing floor for 72 hours, and the vats are flooded with water to make the last larvae crawl off. At Mission, the rearing vats are moved over the water grates; at Tuxtla Gutiérrez, since the vats are fixed in place, the water is turned on through a series of valves. Larval collection continues until most larvae have left the vat; this occurs about 80 hours after their introduction. The cellulose acetate mats used at Mission are vacuumed dry and hung over rails to collect the final few larvae; at Tuxtla Gutiérrez, the cotton linters are pressed dry. In both cases, the support media are finally disposed of by heat treating or burning. Larvae collected at the water-larvae separator are placed in pans (same as starting pans) containing about 3.5 cm of hardwood sawdust at a rate of 3 liters/pan for pupation. These pans are placed on a monorail and conveyed to the pupation room. The larvae and sawdust remain in the pupation room for 16 hours at 26° C and 75% relative humidity.

Pupal collection and handling

Pupae being removed from the pupation room are separated from the sawdust by a shaker-screen separator. The sawdust is reused, and the pupae are transported to the pupal holding area by a slow-moving (1 m/min) conveyor belt (0.5 by 20 m). The pupae and residual larvae

are passed under a lighted passageway, and the larvae exit through the belt for a further pupation period of 4 hours and are then screened again. Pupae leaving the end of the belt are collected in 2.5-liter batches in screened-bottom metal trays and placed in the pupal holding room. Those pupae to be used for colony maintenance are collected at this point. Those pupae to be irradiated are placed in the pupal holding room at 26° C and 75% relative humidity for 5 days before irradiation.

Irradiation and packaging of pupae

Pupae are irradiated as near emergence as possible to prevent damage to the flies. The pupae are placed in a 5.1-liter perforated metal cylindrical canister (12.7 by 50.2 cm) and are irradiated in the Husman irradiator (Isomedex, Parsippany, N.J.), which uses Ce 137 as the radioactive source. The pupae receive a minimum dose of 5 krad to insure sterility. After irradiation, pupae are transported to the field-operation section for packaging before dispersal. At this point, samples of each canister are taken for quality control of the irradiation process.

Controlling Product Quality and Production Yield

To make sure that the screwworms produced will perform as expected after they are released, quality is assessed in several phases of the rearing program. The quality of the insects being released is evaluated; as part of this product testing, the irradiation process is routinely assessed to insure that the released insects are sterile. The quality of the insects being used to maintain the stock colony is also tested. And the quality and quantity of larvae produced in the starting room is evaluated to make sure that this vital part of the mass-rearing process is working properly.

Quality of mass-produced screwworm flies

Various evaluations are routinely performed to assess the quality of mass-produced screwworm flies. Results from these evaluations are used to adjust production handling and distribution procedures to insure that high-quality sterile flies are released. Pupal samples from each shift (taken during the irradiation process) are weighed. The pupal cases are removed to reveal the state of biological development of the flies. This test indicates whether the pupae have developed to within 24 hours of emergence at the time of irradiation. If so, and if the emergence span (hours taken for 90% of the flies to emerge) is known, the proper time of irradiation can be set. Early irradiation reduces emergence and longevity and affects flies in other

undesirable ways. Late irradiation does not allow enough time for packaging, handling, and transportation to the various areas for release and results in reduced emergence and lifespan. An emergence span that requires more than 48 hours means the flies are not uniform and requires changing of the irradiation schedule.

Longevity indicates how long the flies will survive when released. Longevity after release can be affected by irradiation (particularly if not timed properly) and length of time in colonization as well as natural conditions such as temperature, humidity, and availability of food and water. Longevity is determined by hatching the flies at 27°–28° C in a cage containing food and water and then computing the percentage of flies still surviving after 14 days (normally 70%–80% for mass-produced sterile flies).

The quality of the flies subjected to the release procedures is evaluated by sampling the flies (one box per planeload) before and after the distributing plane returns. The emergence percentage and the percentage that fly provide data for evaluating the effects of handling, storage, and dispensing. Agility tests have also been used to assess fly quality. In these tests, the flies are allowed to emerge in a confined space, and the rate at which they disperse is determined. Results from these tests have been highly variable, and efforts are being made to improve the techniques to provide reliable data on the dispersion ability of sterile flies.

Trap-back studies similar to that described by Ahrens et al. (1976) have been useful in evaluating fly quality. These evaluations are particularly valuable in assessing new strains or when serious problems occur in the eradication program. These tests involve releasing marked flies and determining migration, survival, and other quality attributes by computing the percentage trapped at various distances from the release site. These tests provide data on movement and survival of mass-produced screwworm flies and candidate strains to be released. Such tests require much personnel and time for adequate execution, so they are not routinely performed.

A good test of the quality of released sterile flies is obtained by sterility evaluations similar to that reported by Coppedge et al. (1980). These tests are conducted on production and candidate strains to assess their migration, survival, and mating capabilities. The tests determine how much the fertility of egg masses collected in test areas has been reduced. Strains are released in areas of high occurrence of screwworm infestations. Traps are deployed and trap-catch data are collected to determine the migration and survival of the released flies and occurrence of wild flies. Sentinel sheep pens, containing two to three sheep with artificially inflicted wounds, are maintained; eggs laid on the wounds are collected; and the per-

centage of fertility is determined. At the same time, egg masses are collected from animals in the area that have been wounded during ranching operations; the percentage of fertility of these eggs is compared to the percentage determined for eggs taken from the artificially wounded sheep. Fly quality can be measured by comparing the results from the test area to those obtained in control areas or to those obtained from a different group or strain of flies released in the same area. Changes in patterns of fly catches and sterility percentages also indicate changes in fly quality. This type of evaluation is being used in the Sinaloa State of Mexico to assess the quality of flies being mass-produced and released.

Whitten (1980) has used the isozyme technique to assess the quality of various candidate strains of flies to be used in mass production. Bush et al. (1976) suggested that the electrophoretic examination of the isozymes of α -glycerophosphate dehydrogenase could be used to determine changes in the genetics of mass-reared flies. This technique has been used in the Mission laboratory to assess the quality of flies and particularly to monitor the changes occurring during long-term colonization. Presently, this technique is being used in the mass-rearing facilities as another criterion for assessing fly quality.

Goodenough et al. (1978) used an electroretinographic technique to evaluate visual sensitivity of four strains of screwworm flies. They found that the visual sensitivity and the distribution of visual responses of newly colonized strains were nearer than those of older strains to those of native wild flies. So, visual responses can be used to assess the quality of mass-reared flies and to evaluate the degradation of the flies during long-term colonization. These evaluations have been started by the mass-production facilities to assess fly quality.

Quality of the irradiation process

Assessment of the irradiation process is made from 15 pupae randomly selected from each group being irradiated. After adult eclosion, the flies are allowed to mature and mate; any later sign of oviposition or ovarian development indicates the irradiation dosage was not ample for female sterilization. But the female requires about twice the amount of irradiation for sterilization as the male. Male screwworm flies require about 2.5 krad for sterilization (Bushland and Hopkins 1953); so the minimum dosage from the irradiators is set at 5 krad. Although evaluating ovarian development is a possible measure for quality control, it is a time-consuming procedure and requires skill in distinguishing various stages of ovarian development. The technique does not provide on-the-spot assessment of irradiation quality.

Quality of the adult fly colony

Quality of the brood colony is assessed by the volume and percentage of hatch of eggs harvested from each colony cage. When problems occur with oviposition, the rate of sexual maturation in the colony is determined by dissecting samples of females at various stages. But this procedure is limited by available personnel and the quantity of flies that need to be examined.

Larval quality and yield

The quality of the larvae from the starting room is assessed by larval weights, which indicate the quality of the starting-room techniques. A larval weight of 6 mg or more is desired at the end of the starting period to insure proper growth under mass-rearing conditions. Alterations in larval density in the starting pans and modifications of the larval diets affect larval weight. Larvae resulting from 7 g of eggs per pan reach a weight of 6-7 mg at the end of 40 hours of growth after hatch. The weight can be increased to 7-8 mg by reducing the quantity of eggs per pan to 5 g and to 10-12 mg by also increasing the blood level in the diet to 8% and using 3% dried nonfat milk instead of calf-milk replacer (H. E. Brown, unpublished data).

The quality of the larvae produced in the mass-rearing process is also assessed by larval weights coupled with larval yields. These measurements give a good indication of the overall success of the mass-rearing process. Attempts are made to keep larval weight above 60 mg at the end of the rearing process. Alley and Hightower (1966), and Hightower et al. (1972) showed that larger male flies have a higher mating frequency and that flies resulting from larvae weighing less than 56-60 mg are inferior and cannot compete with larger flies. This minimum has been maintained as a standard over the years of production. When a strain has been mass-produced for several months, the larval weights decrease; but, after introduction of new strains, larval weights increase in most instances. The DE-9 strain (a strain produced from egg masses collected near Aldama, Tamaulipas, Mexico), which was recently introduced into the rearing program, produces weekly average larval weights of 72.8 mg each (D. D. Wilson, personal communication). This is the largest average larval weight that has been produced in the program's existence. Larval weights from the previously produced Aracruz strain were averaging slightly above the 60-mg minimum just before the introduction of the DE-9 strain.

Larval weight is also used as the criterion for evaluating various experimental conditions and diets for possible use in the mass-production program. If the new technique or component being tested produces larvae as large as the

standard, then it is considered suitable for mass production of screwworms. Although larval weight may not be the best criterion for assessing quality, it is the best within the limitations of the current program's facilities.

Another way of insuring that the production system is working properly is to assess yields of larvae and pupae. Predicted mass-production levels are based on experience, which has shown that 7 g of eggs should yield 12-13 liters of larvae, assuming that proper techniques are followed and that egg hatch is 95% or better. A reduction in yield may indicate low fertility, problems with rearing techniques, or low quality of dietary ingredients used in the nutrient medium. The cause of the reduction should be found and remedied.

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Improved Techniques for Mass Rearing *Anopheles albimanus*

By Donald L. Bailey and J. A. Seawright¹

Introduction

Anopheles albimanus Wiedemann is an important vector of human malaria over most of Central America and part of South America. Rozeboom (1936) was the first to colonize *An. albimanus*, and few improvements to his system were reported until Ford and Green (1972) published techniques developed during their 2-year study. In the early 1970's, the U.S. Agricultural Research Service's Insects Affecting Man and Animals Research Laboratory at Gainesville, Fla., began intensive studies on the feasibility of releasing sterile males for the control of *An. albimanus* in El Salvador. Not until then was there a need for further improvement of the techniques for mass producing this species. This paper describes all the improvements made since that program began.

Colonization

When Rozeboom (1936) established a colony from field-collected larvae supplemented with field-collected adults, he had problems with high adult mortality and low oviposition rates. And Dame et al. (1974) reported a very slow rate of colonization in a colony of *An. albimanus* from about 11,000 adult females collected from stables in El Salvador. They attributed this slow colonization to a low oviposition rate caused by a low level of insemination of females in filial generations.

During the recent pilot test of sterile-male release in El Salvador, the field performance of mass-reared sterile males (first colonized in 1975) had to be assessed periodically by comparison with a recently colonized field strain. So, to avoid the colonization problems reported by these previous researchers, Bailey, Lowe, and Kaiser (1980) developed an improved system for rapid colonization of *An. albimanus*. This new system required a minimum of effort and material.

Researchers collected adult females from a stable for 10 days. Each day they placed 50 of the collected females in 5-dram plastic vials containing 5 ml of water infused with

a small amount of liver powder and dried yeast. The vials were held for 4 days; then the eggs produced in each vial were counted. This system was compared with one where 50 females were placed in a cage (61 by 61 by 61 cm) each day for 10 days; the number of eggs produced was counted. With both systems, 500 adult female mosquitoes were used. The eggs collected from the vials and from the cages were reared to the pupal stage and placed in separate adult cages. Eggs were collected from the F_1 adults that emerged, and the numbers were calculated. The 500 parent females in vials produced 37,438 eggs with 67% hatch. The F_1 adults produced 207,359 eggs with 63% hatch. This was a 550% increase and indicated that more than 1 million F_1 eggs would have been possible. The 500 parent females in cages produced only 334 eggs, and the F_1 adults did not oviposit.

Genetic Sexing System

The success of the sterile-male method depends on the efficient distribution of sufficient numbers of competitive, sterile males into the habitat of the target species. A sound system must be available for the mass production of sterile males. For the pilot test with *An. albimanus* in El Salvador, an efficient method of separating males and females was also needed. (Since females of *An. albimanus* are potential malaria vectors, their release must be limited even though they are sterilized).

Dame et al. (1974) reported a mechanical method of separating males and females based on pupal size. Lowe et al. (1981) tried to use a membrane filled with malathion-laden blood for the preferential killing of adult females. Neither method was satisfactory for a large pilot project. The mechanical method caused significant losses (up to 40%) of males, and the membrane method was a problem because of the damage done to the insects during the handling of large numbers (about 1 million/day) of adult mosquitoes. So, we developed a genetic method for the preferential killing of females. We knew that if such a genetic method were effective during the egg or first larval stage, the cost of producing sterile males could be reduced by about one-half because twice as many males could be produced with the same space, food, and personnel.

The genetic sexing system that we synthesized used *pro-poxur susceptibility* (*pr^s*) as a conditional lethal (dies

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when treated with propoxur²) trait, a *T(Y;2R)* translocation, and an *In(2R)* inversion. The locus for *propoxur resistance* (*pr*^r), which is dominant, is on the right arm of chromosome two; this allele was linked to the Y chromosome via a radiation-induced translocation (an interchange of chromosomal pieces between nonhomologous chromosomes). We induced six different *T(Y;2R)* translocations. Since crossing over occurs in both sexes of *An. albimanus*, recombinant, resistant females were present in each generation. To suppress recombination, we irradiated males of the translocation stocks to induce an inversion (which reverses a section of a chromosome). Nine genetic sexing strains with <2.5% resistant, recombinant females were detected (Kaiser et al. 1978, Seawright et al. 1978). One of these strains, MACHO, was then produced at the mass-rearing plant in El Salvador. After a few problems in the early phase of implementation (Bailey, Lowe, et al. 1980), MACHO was a complete success. We were able to kill the females of the strain by treating the eggs.

Before the MACHO strain was available, we were releasing an average of 170,000 sterile males per day in El Salvador. The average number of males produced was much greater than 170,000, but inefficient separation methods drastically reduced the number available for sterilization and release. With the MACHO strain, the average release during the last year of the study was 954,400 males per day, and these males were produced with about the same resources as the 170,000 daily average for the regular strain. Also, 99.9% of the mosquitoes produced for release were males.

The synthesis of the MACHO strain is a good example of the way genetic principles can be used in the mass production of insects. In our case, we were attempting to maximize the production of sterile males, but selective breeding schemes can be used to enhance the mass production of insects for any purpose. In fact, many of the problems encountered in mass-production facilities can be solved by genetic approaches.

Production

Production of adults

For the pilot test in El Salvador, we produced about 1 million MACHO sterile males each day. The adult cages and the stocking techniques used to mass-rear *An. albimanus* were those reported by Bailey, Lowe, et al. (1980). They used 126 cages (61 by 61 by 61 cm), made of aluminum window-screen framing and covered on four sides with nylon screen (8 mesh/cm). The inside surface of

the top and bottom of the cage was made of white Formica for an easily cleanable surface. Access into the cage was provided by a sleeve made of a 75-cm length of 25-cm-diameter surgical tubing with one end attached to a 30- by 60-cm opening in the front. At first, the cages were stocked with about 6,000 pupae; then, about 4,000 pupae were added every 2 days for 1 month. After a month in production, each cage was removed, cleaned, and restocked. Four cages were cleaned and restocked each day to minimize the number out of production at one time.

The most important recent advance in maintenance of adult colonies has been the development of a system for feeding of preserved bovine blood through natural animal membranes (Bailey et al. 1978). For our colony maintenance, 61 liters of fresh blood were collected 3 days a week, defibrinated mechanically, and stored in a refrigerator at $5^{\circ}\pm 2^{\circ}$ C. Blood that was to be used for feeding was removed from the refrigerator and placed in condoms made of sheep intestinal membrane (150 ml/membrane). The membranes were closed with clothespins and heated to 44° C in a water bath. A membrane with heated blood was then placed in each of two feeding ports made of 4-inch polyvinyl chloride plastic pipe and tube gauze. The blood was removed, reheated, and returned to the ports twice during the day for a total of three feedings (at 0800, 1100, and 1400 hours). Then the membranes were discarded. Cotton pads saturated with 10% sugar water were provided at all times in two other feeding ports. These ports eliminated the need for entering the cages for feeding, thus minimizing the escape of adults. The use of membranes to feed adult *An. albimanus* has eliminated the need to maintain about 40 rabbits for feeding the adult colonies. At first, a reduction in egg production was associated with the preserved blood; but, in time, egg production returned to a normal level.

Production of eggs

Eggs deposited overnight in plastic pans (15 cm diameter and 8 cm deep) containing 2 cm of water (one pan per cage) were collected by pouring the water containing the eggs through nylon screen (12 mesh/cm) to remove dead mosquitoes and other debris. About 90% of the eggs were then poured into a 0.01% propoxur solution and held for 24 hours to kill the females. The remaining 10% were left untreated and used for colony replacement.

In earlier rearing of *An. albimanus*, the eggs were held an additional 24 hours to hatch, and numbers of the hatched larvae were estimated and the larvae placed in rearing trays. Dame et al. (1978) described an improvement in which the eggs were dried after the first 24 hours and samples were measured volumetrically to obtain accurate

²o-Isopropoxyphenyl methylcarbamate.

numbers of larvae in the rearing trays. The eggs were dried in plastic trays (25 by 18 by 7.5 cm high) with a bottom of white nylon cloth by drawing air through the tray with a fan for 30 minutes. Dried eggs were sifted through stainless steel screen (40 mesh/cm) to break up clumps. Then a vibrating device was used to measure them volumetrically into plastic microcentrifuge tubes cut to length to hold the desired quantity.

With the standard strain of *An. albimanus*, we needed 0.085 ml of eggs (average of 6,779 eggs) per rearing tray. But the MACHO strain is naturally 50% sterile (eggs do not hatch) because of the translocation; so we needed 0.17 ml of untreated eggs to achieve equivalent production. Since the females (99.9%) had been killed with the egg treatment, we needed 0.34 ml of treated eggs per sample. These samples were then placed in individual 350-ml Styrofoam cups containing 75 ml water and 1.4 ml of a 2% liver and yeast suspension (1 : 1). These cups were held for 24 hours at $29^{\circ}\pm 0.5^{\circ}$ C for the eggs to hatch.

Excess eggs were stored in 100-ml plastic bottles at 10° C (Bailey, Thomas, et al. 1979), so a stockpile was available for use in case of an unexpected reduction in egg production. Since untreated eggs can be stored for 7-10 days with little reduction in hatch, they can be used for colony maintenance or treated later with propoxur to rear stock for field release. Treated eggs can only be stored for 2-3 days without reduced hatch.

Production of larvae

The larval rearing trays used for mass production were made of ABS plastic (56 by 43 by 7.5 cm high), and each contained 3 liters of water. A precise temperature-control system using electrical heating tapes and electronic proportional controllers (Dame et al. 1978) was devised. The shelves holding the rearing trays were 15 m long and arranged in banks of nine. Each shelf had one 30-m heating tape that ran the full length of the shelf and doubled back again; the two strands were 30 cm apart. One controller regulated the tapes on three shelves; so three controllers were used for each bank. With this system, the water temperature in the rearing trays could be maintained at $29^{\circ}\pm 0.5^{\circ}$ C.

The trays containing water (300 containing propoxur-treated eggs and 104 containing untreated eggs) were placed on the shelves for 24 hours so the water temperature would stabilize at $29^{\circ}\pm 0.5^{\circ}$ C before the newly hatched larvae from the hatch cups were poured in. Just before the larvae were introduced, 150 ml of a liver powder, yeast, and 40% protein hog supplement (1 : 1 : 1) food suspension (2.25 g dry ingredients) was added to each tray. The larvae received an identical feeding 72 hours later. On the next 2 days, each tray received 150

ml (3 g dry ingredients) of a suspension of hog supplement alone.

Production of pupae

On the day after the last larval feeding (day 6 after the larvae were placed in trays), pupae were removed. The remaining larvae were consolidated (four trays in one) and returned to the rearing shelves with the original water (Fowler et al. 1980). The next day, pupae were again removed, but this time any remaining larvae were discarded. The pupae were removed from the rearing trays by a system, described by Bailey, Lowe, et al. (1980), adapted from Weathersby (1963). A mixture of larvae and pupae was placed in a large plastic funnel that contained cold water (10° C) and had a shutoff valve attached to the outlet. Larvae sank to the bottom in the cold water, and the pupae remained on the surface. When the valve was opened, the larvae passed through first and were collected on a screen; then they could be returned to the rearing trays or discarded. The pupae were then collected on a screen and used to restock rearing cages or were sterilized for field release depending on whether they had been treated with propoxur. The consolidation of trays of larvae greatly increased efficiency by increasing the number of trays that could be set in a given space and by decreasing the number of trays handled during the second harvest. The quality of the insects was not reduced by this system.

Production Management

In our rearing program, we had to monitor environment, maintain specified production levels, and insure that our product insects met the standards set for their use in colony maintenance and sterile-male release. The laboratory staff in El Salvador consisted of two entomologists, a program assistant (who collected, compiled, and organized the data), an administrative assistant (who organized work schedules and coordinated responsibilities of the technical staff), and 26 technicians. The technical staff was divided among five sections responsible for adults, eggs, larvae, pupae, and special research. Each section had a supervisor and an assistant supervisor and the necessary personnel to perform the duties. Work schedules were arranged so production was possible for a full 7-day week. The supervisor of each section was responsible for daily reports on that section's function.

Environmental monitoring

A hygrothermograph was operated constantly in the adult colony. Each day, the supervisor of the adult section reported the temperature and humidity at 2400, 0800, 1200, and 1600 hours. He also reported the temperature of the refrigerator where the preserved blood

for feeding the adults was stored. The supervisor of the egg section reported the temperature of the water in which the eggs were incubated and also the temperature in the refrigeration unit where the extra dried eggs were stored. The supervisor of the larval section reported the water temperature in the rearing trays at each level for all the rearing shelves at 1000 hours each day. The supervisor of the pupal section periodically checked and reported the temperature of the cold water for pupal separation. These daily reports were absolutely essential to alert the entomologist in charge of any malfunction in the environmental control equipment. If corrections were made soon enough, there was usually little or no loss in production.

Maintaining production levels

The supervisor in each section was also responsible for reporting the production levels in his section each day. For the adult section, this report included the total number of cages of adults, number of cages stocked with pupae, number of pupae per cage, and number of cages cleaned and restocked. The egg section reported the number of cages producing eggs, total egg production (ml), average egg production per cage (ml), number of hatch cups set, amount of extra eggs stored (ml), and number of rearing trays set. The supervisor of the larval feeding section reported the number of trays receiving food (first through fourth feedings) and the total number of bad trays (those trays with total or almost total mortality by the fourth day). He was also responsible for collecting random samples of the food mixture during feeding and for running sedimentation tests on the samples to determine whether the food was being formulated and mixed properly. The supervisor of the pupal-handling section reported the total number of trays harvested (first and second harvests), the average pupal production per tray (based on 3 randomly selected trays from each harvest each day), the relative size of the pupae (average number of pupae in 3 samples of 1 ml each), and the sex ratio from each harvest (based on 3 samples of 100 pupae each).

Quality Control

High yields are useless if the insect does not survive, or if it does not mate once released. In the El Salvador project, quality was judged with tests done in the laboratory and in the field.

Laboratory tests

Because a genetically altered strain was being used, certain checks were made to insure the integrity of the genetic sexing system. For example, the percentage of egg hatch (treated and untreated) was checked daily. The

expected untreated hatch was 50% (because of the translocation) and the expected treated hatch was 25% (because of deaths among females). Another indicator was sex ratio, and we made a daily check of the sex ratio of the pupae reared from propoxur-treated eggs. If the system was working properly, the pupae from these trays were about 99.9% males. But about 0.1% resistant females were produced through genetic recombination in each generation in the MACHO strain, so the number of these females was expected to increase over time. Also, at weekly intervals, adult males and females that had not been treated in the egg stage were exposed to residues of 0.1% propoxur on filter paper for 1 hour, and mortality was recorded. The data were used to monitor levels of resistance and susceptibility in the males and females. When the frequency of resistant, recombinant females became excessive, they had to be purged by crossing propoxur-resistant translocation males with females that were totally susceptible. This nucleus colony was then increased for three or four generations until it was large enough to be used to replace the old colony. Also, if the MACHO strain was accidentally contaminated with normally susceptible stock, the increase of susceptible males accelerated rapidly.

Each day, a sample of 300 pupae from the rearing trays was placed in a cage, adults were allowed to emerge for 48 hours, and the percentage emerging was recorded. The adults were then constantly provided with 10% sugar water on cotton pads and held at $27 \pm 2^\circ \text{C}$, and the percentage of the males and females surviving after 7 days was reported. This system indicated whether the insects being produced were healthy. Also, some males that had been sterilized for field release and some nonsterile males were crossed with normal females each day. The percentage of egg hatch from those crosses was calculated to determine how effective sterilization techniques had been.

Field tests

Adult emergence in the field was monitored daily. But the most important test—the determination of the competitiveness of the laboratory-reared, genetically altered, chemically sterilized male—could only be made by using comparative field releases. For this, we selected part of the 30-km² test area that had an extremely small natural population of *An. albimanus* (Kaiser et al. 1979, 1981). The comparison had the sterile MACHO males and males of a recently colonized field strain, CAMPO, compete for released CAMPO females. The releases consisted of two replicates, each at a ratio of about five sterile MACHO males to one CAMPO male to one CAMPO female. About 10,000 females were released per replicate. Pupae were packaged for transport to the release site according to

the method described by Bailey, Lowe, et al. (1979). Calf-baited traps (Lowe and Bailey 1981) were placed about 125 m north and south of the release site. A calf was placed in each trap at 1800 hours and removed the next morning. Adult *An. albimanus* were collected each day from each trap, and the females were returned to the laboratory. Males were excluded to prevent further matings in the cages.

Females were held in the laboratory for 2 days and then immobilized in a coldroom and placed in 5-dram plastic vials, one female per vial. Five ml of water was added, and the females allowed to oviposit. After 5 days, the eggs were checked for hatch to determine whether the mating had been with a sterile or fertile male. Competitiveness was determined by the formula (Fried 1971) that

$$C = S/N(\text{calculated}) \div S/N(\text{actual}),$$

where $S/N(\text{calculated})$ = the ratio of irradiated males to normal males that will give an expected percentage of sterility if mating $N\varnothing \times N\sigma$ gives >5% hatch and if mating $N\varnothing \times S\sigma$ gives 0%–5% hatch;

and $S/N(\text{actual})$ = the actual ratio used experimentally.

The average mating competitiveness of the MACHO males was 78.5% of that of the CAMPO males; their average dispersal ability was 74%. So the males of the genetic-sexing strain could disperse and could induce high levels of sterility in indigenous populations.

Conclusion

The rearing techniques described in this paper have been refined through a great amount of research effort. The requirements for each life stage in the mass production of *An. albimanus* have narrow limits, and each is critical to the successful rearing of this species. One factor is not necessarily more critical than another, because any single weakness or deficiency in the system can cause the entire system to fail.

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Some Systems for Production of Eight Entomophagous Arthropods

By E. G. King¹ and R. K. Morrison²

Introduction

Arthropod rearing is basic to most entomological endeavors, especially biological control. For example, importing entomophagous arthropods (predators and parasites, the "natural enemies") for establishment often requires that these organisms be reproduced for several generations in quarantine before release. Even after establishment, large-scale production in insectaries may be necessary to expand the entomophage's geographic distribution. Likewise, conduct of today's integrated pest management programs requires detailed knowledge of the entomophage's biology, behavior, and effectiveness. Availability of these organisms for research often depends on our ability to maintain colonies of them in the laboratory. Finally, control of pest arthropods by augmenting their predators and parasites through periodic releases also requires large-scale production. So specialized equipment and unique techniques have been developed for producing hosts, because artificial diets and in vitro rearing methods have generally not been developed for large-scale production of predators and parasites.

Methods for rearing many kinds of entomophagous arthropods have been reviewed by Finney and Fisher (1964) and Morrison and King (1977), as have facilities (including quarantine) and specialized equipment for rearing entomophages and their hosts by Fisher and Finney (1964) and Leppla and Ashley (1978). But evaluating a rearing program requires consideration of many factors besides production capability, especially product quality (Boller and Chambers 1977).

Here we examine several systems currently being used for producing entomophagous arthropods, and we comment on the strengths and weaknesses of each system. We will mainly discuss these predator and parasite species: *Aphytis melinus* DeBach; the common green lacewing, *Chrysopa carnea* Stephens; *Cryptolaemus montrouzieri* Mulsant; *Encarsia formosa* Cahan; *Lixophaga*

diatraeae (Townsend); *Spalangia endius* Walker; *Phytoseiulus persimilis* Athias-Henriot; and *Trichogramma* sp. These are being mass-reared and used for control of their natural hosts by augmentative releases in various parts of the world. We also briefly discuss the use of natural or unnatural hosts (those not normally attacked in nature, but suitable in the insectary) for laboratory rearing of entomophages and survey recent accomplishments in the development of artificial diets for them.

Systems for Producing Entomophages

Production of *Aphytis melinus*

A. melinus is reared continually and released regularly for control of the California red scale, *Aonidiella aurantii* (Maskell), in the citrus-growing areas of California (Pennington 1975). DeBach et al. (1950) initially demonstrated the potential of controlling California red scale by augmentative releases of *A. chrysomphali*, and DeBach et al. (1955) further defined it. After the field demonstration, procedures were developed for mass producing *A. chrysomphali* on California red scale fed potato tubers. But the method developed by DeBach and White (1960) that uses the unnatural host—oleander scale, *Aspidiotus nerii* Bouche—for production of the California red scale parasite, *A. lingnanensis* Compere, was better and is still used by at least two commercial insectaries in California. The imported species *A. melinus* is now reared and released instead of *A. lingnanensis*, but the procedures remain essentially the same as those described by DeBach and White (1960).

A. melinus is produced (fig. 1) on a uniparental strain of the oleander scale fed banana squash, *Cucurbita maxima* Dend. Each day, crawlers (the immature mobile stage of oleander scale) are implanted on fresh squash. These crawlers are collected from squash that have been infested for 58–73 days. Infested squash about 73 days old are offered to the parasite in oviposition-collection units supplied with honey, the food for the adult parasites. A specified number of freshly emerged and collected adult parasites is placed on the unparasitized squash, and the oviposition-collection unit is closed. After 24 hours for oviposition, the parasites in the unit are anesthetized, removed, and prepared for field release. The now-parasitized squash are moved from the unit to storage racks for parasite development. Removing the squash also allows continual use of the oviposition-collection

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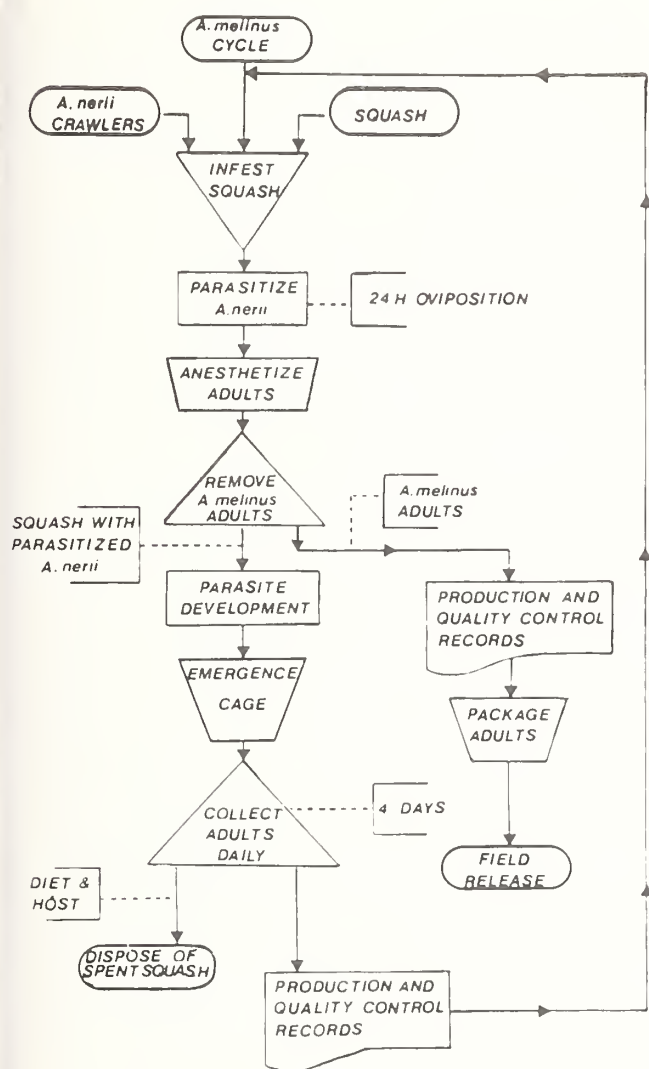


Figure 1.—System for producing *A. melinus* (parasite) on *A. nerii* (host) fed banana squash.

unit. About 2 or 3 days before emergence of adult parasites, the squash are placed back into a prepared unit, and the parasites are collected each 24 hours throughout the emergence cycle.

Extensive tests were conducted in selecting the host (oleander scale) and its food source (banana squash) by DeBach and White (1960) and Finney and Fisher (1964). No special problems remained after this host was chosen, but careful attention has to be given in the selection, storage, and handling of the squash. DeBach and White's (1960) production program is sound and functional, though rearing procedures could undoubtedly be auto-

mated, particularly in handling of materials. The system has had no major engineering input, probably because it has production facilities at each of the major citrus-growing areas instead of one large facility at a central location.

Apparently, rigid procedures have not been developed and implemented for monitoring and maintaining the quality of *A. melinus*. But genetic variability in the genus *Aphytis* is fully recognized in California and has been exploited by researchers in establishing species of *Aphytis* effective against the California red scale. And DeBach and Hagen (1964) reported that strains of *A. lingnanensis* had been selected for increased tolerance to cold and heat. Use of these strains could improve their effectiveness in augmentation programs. Basic measurements of production efficiency, which can warn of possible breakdowns in insect quality, are built into the production system. And the sex ratio is routinely measured.

Production of *Chrysopa carnea*

Rearing and periodically releasing *C. carnea* to prey on many insect pests has been very effective (Beglyarov and Smetnik 1977, Ridgway et al. 1977). Its effectiveness may be improved by use of supplementary foods containing materials that attract, retain, and stimulate the adults to oviposit (Hagen and Hale 1974). Mass production of *C. carnea* is limited by its costliness, though several artificial diets have been developed for rearing the larvae (Singh 1977), and methods for encapsulating some of these diets have been developed by Hagen and Tassan (1965) and Martin et al. (1978; see also Agricultural Research 1971). But eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier), or eggs of other hosts, remain the preferred larval food source (Beglyarov and Smetnik 1977, Morrison and King 1977) because they provide the best balanced and most available diet. An inexpensive, highly acceptable adult diet that induces and maintains high rates of fecundity has been developed by Hagen and Tassan (1970). But the Wheat in this diet is no longer available and has been replaced with a comparable product—Formula 57 available from CRS Co., St. Paul, Minn. (K. S. Hagen, personal communication).

The basic technique for production of adult *C. carnea* from eggs (fig. 2) evolved from the system first reported by Finney (1948), later improved on by Rincon-Vitova Insectaries, and further developed by Morrison and Ridgway (1976) and Morrison (1977). Food (frozen Angoumois grain moth eggs) is mixed with fully embryonated *C. carnea* eggs and distributed into multicell rearing units. The cells are covered on both sides with organdie, which the hatched larvae can feed through during three later feedings. Morrison (1977) reported coating glass, cut the same size as the unit, with a honey-water

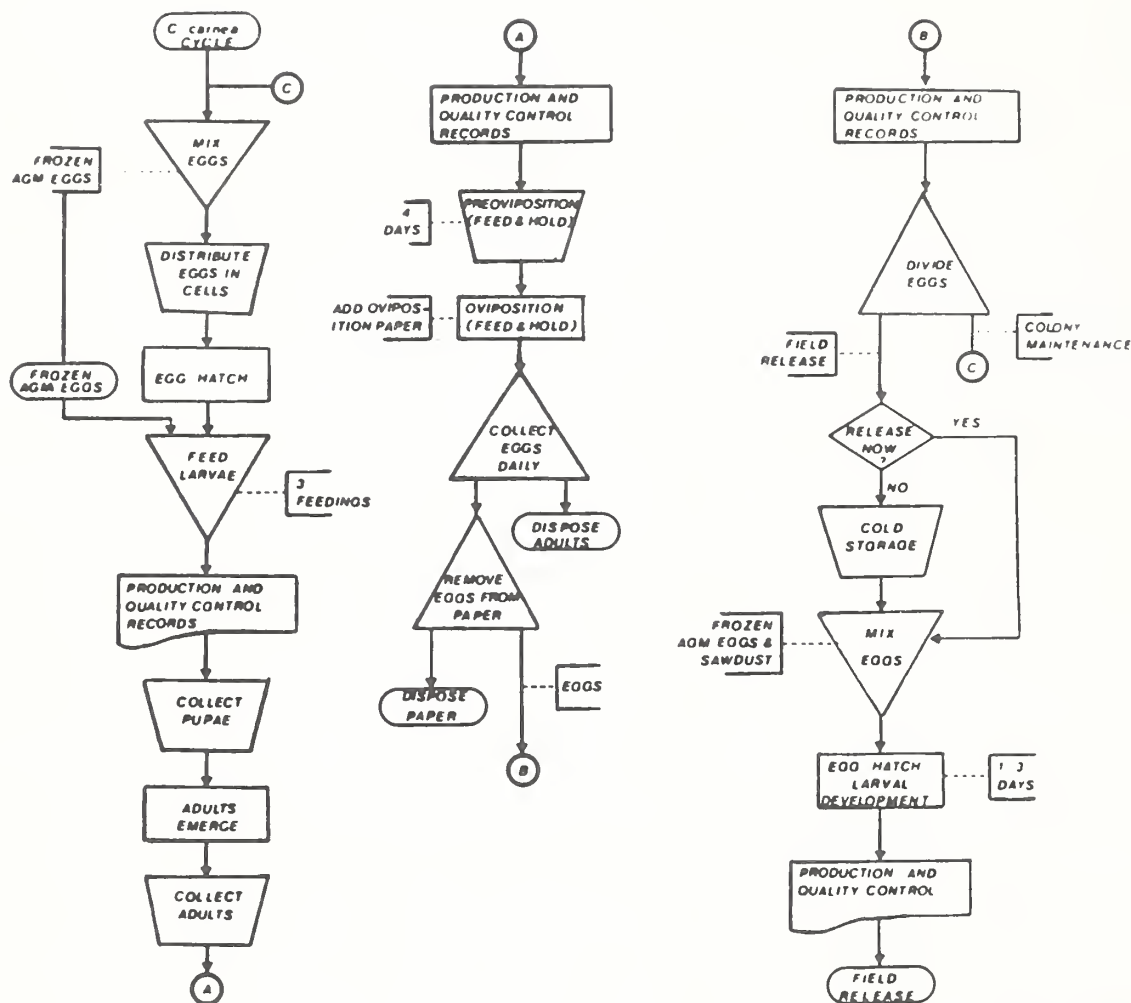


Figure 2.—System for producing *C. carnea* (predator) on frozen Angoumois grain moth (AGM) eggs.

mixture (1 : 1) and Angoumois grain moth eggs for feeding the larvae. This plate is inverted over the cloth.

After pupation, the organdie is removed from the cells, and the pupae are placed together for emergence. As the adults emerge, they are collected daily, held in preoviposition units, and supplied adult diet and water for 4 days. Thereafter, the units are lined with paper on which the adults lay their eggs. This paper is coated with the adult diet, a combination of Formula 57, sugar, and water. (A suitable diet must be continuously available to maintain high fecundity.) When 0-to-24-hour-old eggs are present, the paper liners are then removed and replaced daily. Morrison and Ridgway (1976) used a vacuum to immobilize the adults and then transfer them to clean units for each change of oviposition paper. The kraft-paper

liners and attached eggs are removed from the oviposition units and held overnight in room conditions (about 22° C) so the eggs will harden. When a hand-held ball of nylon net is wiped over the paper oviposition sheets, the hard nylon threads of the net easily break the egg stalk, and the eggs come off. The collected eggs can be used for maintenance of the colony or for rearing larvae to distribute in the field. For field distribution, the *C. carnea* eggs are mixed with sawdust and frozen Angoumois grain moth eggs; the mixture is held until the resulting larvae are 1–3 days old; and then the larvae are mechanically distributed (Agricultural Research 1972).

Production efficiency is continually monitored, but methods for measuring quality have not been defined. Tests have shown that adult survival and fecundity does

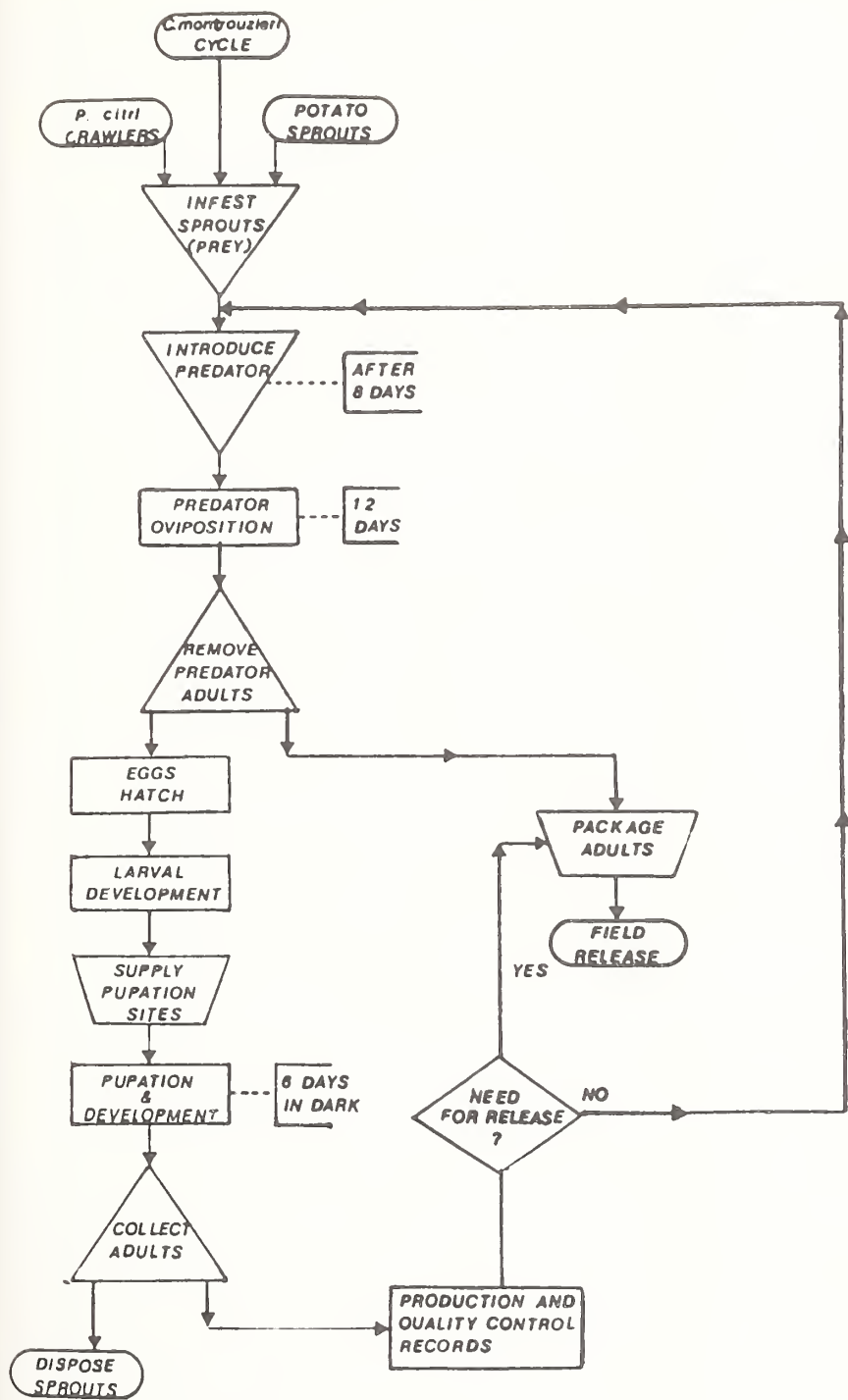


Figure 3.—System for producing *C. montouzieri* (predator) on *P. citrii* (prey) fed potato sprouts.

decrease with increasing time in culture. Also, Jones et al. (1978) found indications that developmental time of immature stages increases, and egg viability, food consumption, and searching ability of *C. carnea* decreases with increasing time in culture. So they recommended that *C. carnea* intended to augment natural populations should not be held in mass culture for more than six generations before release.

Production of *Cryptolaemus montrouzieri*

Armitage (1919) first reported on rearing and release of *C. montrouzieri* for control of mealybugs in citrus. (A complete documentation of the historical development of the use of *C. montrouzieri* is given by DeBach and Hagen 1964.) Beglyarov and Smetnik (1977) reported that *C. montrouzieri* is mass-reared in the U.S.S.R. and "hundreds of thousands" are released annually in plantations of citrus, grapes, tea, and other plants for control of mealybug and scale insects.

C. montrouzieri has been reared on various hosts feeding on several food sources. But rearing methodology (fig. 3), regardless of host and plant-food source, is usually like the system reported by Fisher (1963). The citrus mealybug, *Planococcus citri* (Russo), is mass-reared on potato sprouts grown in subdued light on soil held in wooden trays. After the sprouts are about 4.7 cm tall, mealybug crawlers are allowed to crawl onto freshly cut leafy terminals of *Pittosporum undulatum* Vent. or *Schinus molle* L., which are placed in the trays for about 6 hours. These terminals are then placed among the potato sprouts in darkened rooms, and the crawlers move onto the sprouts as the terminals begin to dry. After 8 days, *C. montrouzieri* adults are placed in the trays where they lay eggs on the potato sprouts and trays for 12 days. Then these adults are collected at opened windows screened with cotton muslin and released in the citrus orchards. Meanwhile, burlap bands are attached to the front of the racks holding the trays as a substrate for pupating beetle larvae, and the holding room is again darkened for 6 days, the window shutter is once more raised, and emerging beetles are collected for release at the cloth-covered window opening.

Since *C. montrouzieri* was imported into the United States in 1892 (DeBach and Hagen 1964), there has been no success in either locating or selecting a strain that can withstand temperatures below 0° C (Beglyarov and Smetnik 1977). We found no reports on how laboratory rearing affects genetic processes or maintenance of desirable characteristics in laboratory-reared beetles.

Fisher (1963) discussed in detail how to select the most suitable potato variety for rearing the citrus mealybug.

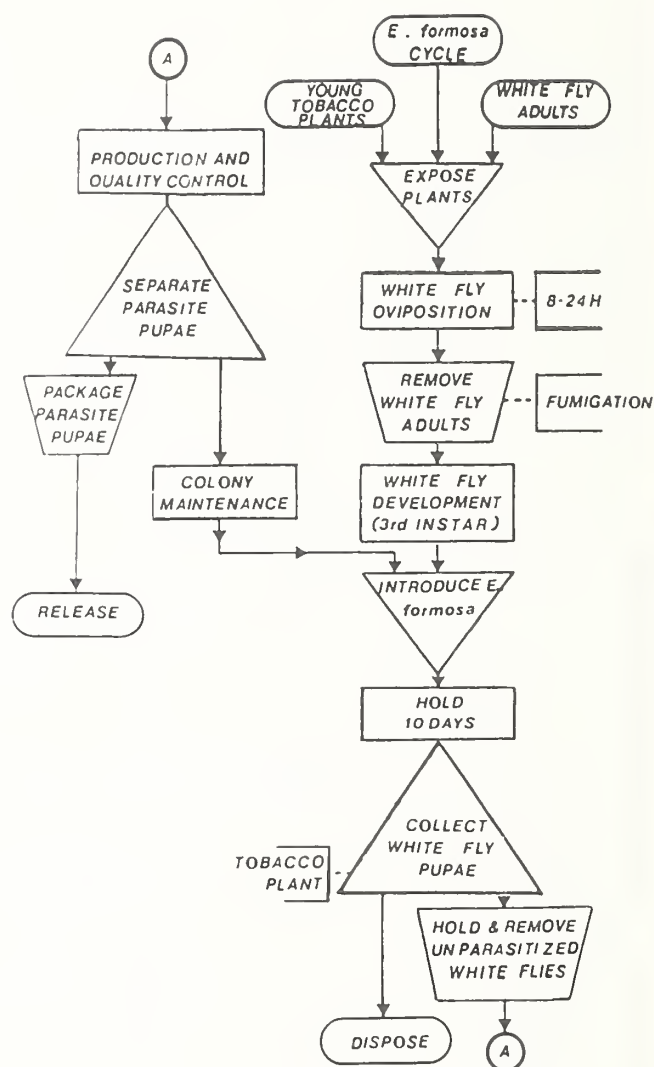


Figure 4.—System for producing *E. formosa* (parasite) on whiteflies (host) feeding on tobacco plants.

Apparently, it feeds on sprouts of some potato varieties more readily than on others, certain varieties are more readily available than others, and some potatoes bruise more easily than others (so they have a shorter storage time). Watering the potato sprouts and implanting crawlers on them must be done carefully. Beglyarov and Smetnik (1977) reported that an artificial diet had been developed for rearing *C. montrouzieri*; this diet might eliminate many problems associated with maintaining the host and its food source. Fisher (1963) did not mention any pathogens affecting the beetle colony; however, contaminants did affect storage time of the potatoes. Many rearing procedures could be mechanized; facilities for

maintenance of the proper environmental conditions could be built; and the handling of materials could be simplified.

Production of *Encarsia formosa*

Hussey and Bravenboer (1971) reported that *E. formosa* is widely used in Europe, particularly England and the Netherlands, for control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). There are two basic techniques for host (whitefly) production; one uses cucumber plants, *Cucumis sativus* L. (Morrison and King 1977) and the other uses tobacco plants, *Nicotiana tabacum* L. (Glasshouse Crops Research Institute 1975, Hussey and Scopes 1977). In the system using tobacco (fig. 4), the tobacco plants are exposed for 8–24 hours to whitefly adults, which lay about 100 eggs/6.5 cm². Then the plants are shaken by hand and fumigated with dichlorvos³ to eliminate the adults; and the egg-infested plants are held until the whitefly reaches its third stage ("ceases to be flat disc and develops elevated sides"). At that time, adult parasites are placed on the plants. Ten days later the parasitized and unparasitized whitefly pupae are brushed from the leaves into suitable containers and held for emergence of the unparasitized whiteflies. (They emerge before the parasites; so they can be easily removed from the parasite culture.) The remaining parasites are used for release in commercial glasshouses and for the reproductive colony. Other methods are available for producing the parasite on tomato plants while the whiteflies are produced on tobacco (Glasshouse Crops Research Institute 1975).

Cucumber plants for producing hosts and parasites are used mainly in the Netherlands. There, plants grown vertically on string are the hosts. At first, about 100 whitefly adults are implanted on the young plants. Then, about 2 weeks later, *E. formosa* is introduced as a mature pupa at about 8 parasites to 10 whiteflies. After 2 more weeks, harvest of the bottom leaves (containing only mature *E. formosa* pupae and emerging adults) begins after inspection shows that some parasites have emerged. Both pest and parasite move up the plant to newly emerged and infested leaves, and this production unit can sustain itself for several months.

None of these studies gave methods for measuring the behavioral quality of the mass-produced parasites. Production is apparently closely monitored. And raising or lowering the temperature can influence synchronization of host and parasite development.

Production of *Lixophaga diatraeae*

Ridgway et al. (1977) report that *L. diatraeae* is reared and periodically released in several Western Hemisphere countries for biological control, mainly of the sugarcane borer, *Diatraea saccharalis* (Fabricius). It can be reared on many lepidopterous larval species (Bennett 1969), but King et al. (1979) reported that a large-scale production system has recently been developed on an unnatural host, the greater wax moth, *Galleria mellonella* (Linnaeus). The method discussed here maintains the parasite on its natural host, the sugarcane borer.

In the system for producing *L. diatraeae* (fig. 5), the adult flies are held in cages covered with gauze. The cages contain food and water. When they are 12–14 days old, the flies are aspirated into a collecting jar containing 1% NaOCl. After the NaOCl is rinsed from the flies, they are blended at 8,500 r/min (revolutions per minute) for 9 seconds in a 0.7% Formalin (formaldehyde) solution so the maggots can be extracted. Passing what is left through a screen separates the maggots from the remaining fly particles. Then the maggots are rinsed, suspended in a 0.15% agar-water solution, and placed in a gridded petri plate where the total maggot number is determined. Afterwards, more agar-water solution can be added to produce the desired maggot density in a given solution. For either the sugarcane borer or the greater wax moth, the larvae are exposed to the parasite maggots during the last stage. The agar-water solution containing the maggots is metered into 30-ml cups containing sugarcane borer larvae and diet. When *L. diatraeae* are reared on the greater wax moth, larvae are placed in trays containing the parasite maggots, which have been poured or air-brushed. Then a screen is placed over the trays; after about 1 hour, corncob grits are added to the trays to absorb excess moisture. On either host, the maggots complete development in 6–9 days, and both hosts and parasite puparia are harvested on the 11th day after parasitization. Puparia are removed with forceps from cups containing sugarcane borer larvae. Puparia formed outside greater wax moth cocoons are brushed from the trays; those retained in the host cocoon are collected by flotation after the cocoon is dissolved in 1% NaOCl. Puparia to be shipped are packed between layers of cotton in cartons and placed in styrofoam boxes containing icepacks that will maintain low temperatures (18°–25° C) in transit. The flies are released in sugarcane fields 4–6 days after emergence (prelarviposition period).

In the system discussed by King et al. (1979) for producing *L. diatraeae*, production was routinely monitored by measuring of adult survival; mating; sex ratio; maggot production; and percentage of parasitization of host larvae, of puparia production, and of fly emergence. No routine methods were developed for monitoring parasite

³2,2-Dichlorovinyl dimethyl phosphate.

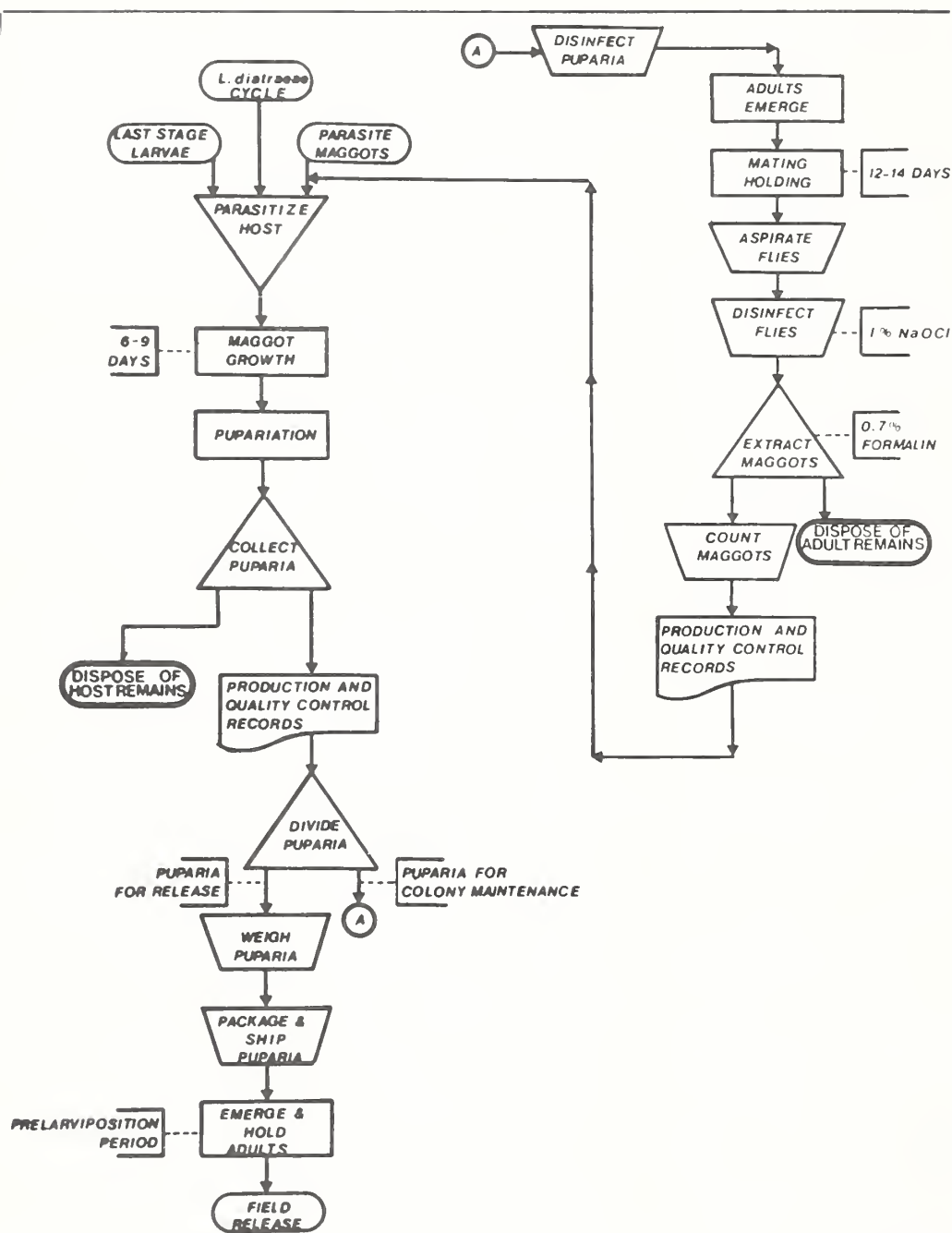


Figure 5.—System for producing *L. diatraeae* (parasite) on its natural host, the sugarcane borer, or an unnatural host, the greater wax moth.

fedest *V. faba* plants are laid on pots of mature *P. vulgaris* that will be a food source and substrate after implantation with *P. persimilis*. In the Netherlands, *T. urticae* are implanted after appearance of the first true leaves (fig. 7). When the second true leaves appear, *P. persimilis* is introduced. Once *P. persimilis* has multiplied and has eliminated the *T. urticae*, the leaves can be harvested and stored at low temperatures (7.3°–12.9° C) or delivered to release spots. Although production records are kept, there are apparently no routines for monitoring the quality of the predator or parasite produced.

Production of *Trichogramma* species

Species of *Trichogramma* are mass-produced and used to control caterpillar pests in the U.S.S.R. (Shcheptil'nikova et al. 1974), China (National Academy of Sciences 1979), and Mexico (Gomez 1975), and also, but not as much, in Western Europe and the United States (Starler and Ridgway 1977). The parasites are typically mass-produced on unnatural hosts and not the target or natural host.

The Angoumois grain moth, *Sitotroga cerealella* (Olivier), is used worldwide, except China, as an insect host for *Trichogramma* because it can be easily and inexpensively mass-produced. In China, most large-scale *Trichogramma* production is on eggs from a silkworm, *Antheraea pernyi* Guérin-Méneville, reared outside on oak trees, *Quercus* spp., grown in field plots. Other insect hosts include the Mediterranean flour moth, *Anagasta kuehniella* (Zeller), in Western Europe and another silkworm, *Samia* (= *Philosamia*) *cynthia ricini* (Boisduval), and a grain moth, *Corcyra cephalonica*, in China. Interestingly, as a byproduct of parasite production, the silkworm cocoons can be used for sericulture. Nevertheless, not all important *Trichogramma* spp. can be reared on silkworm eggs—*T. ostrinaeae* for example—so *C. cephalonica* is also needed in China.

In China, the silkworm reared on oak foliage is harvested from field plots after the larvae have formed cocoons in late summer. The cocoons are stored at low temperatures during the winter and until needed the next year. Cocoons containing female pupae are mechanically separated from those containing male pupae by size and weight (the female is larger and heavier). The female moths are collected as they emerge and passed through a grinder, a process that extracts the unfertile eggs. Screening and decanting the water used in the grinding process separates the eggs from other body parts; the eggs are then centrifuged and dried. Since the eggs are not fertile, they can be stored at low temperatures for several weeks after extraction without embryonation occurring. For parasitization, the eggs are glued to cards or sheets of

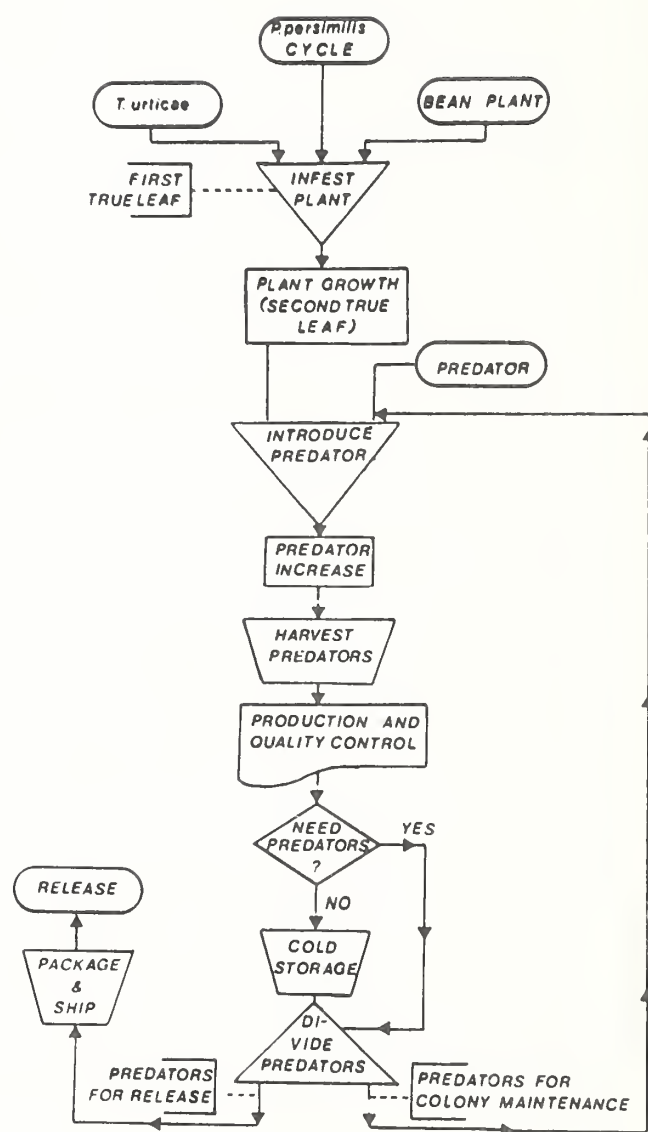


Figure 7.—System used in the Netherlands for producing *P. persimilis* (predator) on *T. urticae* (prey) feeding on bean plants.

paper and exposed to the parasites for 2 or more hours. Typically, unparasitized eggs are placed near a light source 1 or more meters from the reproductive stock of parasites. The parasites are positively phototactic; so they will find the eggs after flying to the light source. After enough parasites are on the host eggs (about one parasite per egg) they are moved to a darkened area to complete parasitization. The parasitized eggs can be stored at low temperatures (5° C) until time for field release.

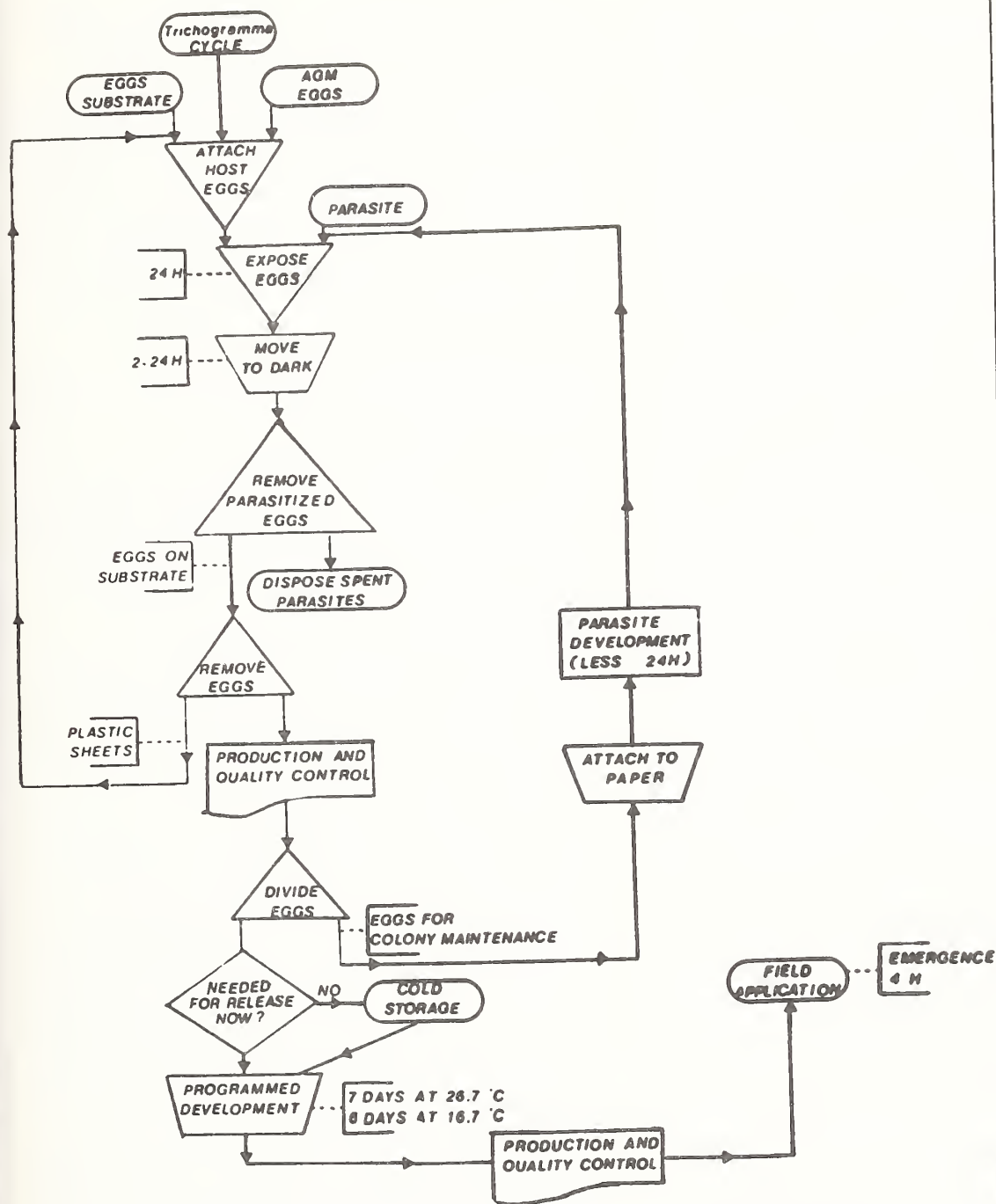


Figure 8.—System for producing *Trichogramma* (parasite) on Angoumois grain moth (AGM) (host) eggs.

The basic system (fig. 8) used for producing *Trichogramma* in the United States (Morrison et al. 1978) and in the U.S.S.R. (Beglyarov and Smetnik 1977) requires exposing Angoumois grain moth eggs to *Trichogramma* adults in a closely confined area. The host eggs are attached to plastic sheets with a fine mist of water, or they are glued to paper. After the desired exposure time has elapsed, the now-parasitized host eggs are removed from the parasite chamber and held for parasite development. Parasitized host eggs can be broadcast on fields if they are attached to plates or point-released if they are glued to paper.

Specifically, after host eggs are stuck to plastic or glass sheets, the sheets are exposed to parasites in a glass-sided, illuminated oviposition cage for 24 hours (Morrison et al. 1978). The sheets containing the now-parasitized eggs are moved to the back half of the oviposition cage, which does not have glass sides and is dark. At the same time, freshly prepared eggs are introduced. After 20 hours, the parasitized egg sheets in the back are removed through a door at the dark end of the oviposition cage. And, while the door is open, specific amounts of parasite pupae due to emerge within 24 hours are placed in the oviposition cage, and similar material placed in the cage 4 days earlier is removed. So a consistent, specific number of ovipositing adults is in the cage. Since *Trichogramma* is positively phototactic, those adults remaining on the host eggs at the dark end of the unit and those emerging from pupae move to the light at the glass-sided end of the unit. Light intensity and diffusion must be adjusted so the parasite will be attracted to the lit end of the cage but not so strongly attracted that it remains on the glass exclusively. Also, glass sides of the cage are alternately lit and darkened so that phototactic parasites will move back and forth across the host eggs. This movement results in a consistent parasitization rate.

The parasitized eggs on the sheets removed from the cage are brushed onto organdie-covered frames for further development at 26.8° C. After enough oviposition stock is reserved, the remaining parasitized eggs are placed in cold storage (R. K. Morrison, unpublished data) or they are cold-programmed for aerial release (Bouse et al. 1978). The temperature-programming system used requires holding the eggs at 26.7° C for 6 days and transferring them on the seventh day to a chamber where they are held at 16.7° C for 6 more days. Over 75% of the *Trichogramma* will emerge from these eggs within 4 hours after application in the field.

Regardless of the system, quality-control procedures are typically production oriented and consist of keeping records on production, parasitization rate, emergence, and sex ratio. Each system does stress the need for replacing the colony every year with field-collected material to avoid release of a parasite that may have reduced effi-

ciency in host searching and parasitization. The Chinese system is unique because it requires the parasites to fly several feet in search of host eggs, so it continually eliminates weak, inactive individuals. Also, in China, laboratory colonies of *Trichogramma* spp. are typically replaced with field-collected parasites after about 15 generations because of deterioration (J. Z. Bao, personal communication). In the United States, selection of a parasite strain that will attack the target host is accomplished by annual colony establishment with parasites collected only from the target host and affected crop. Also, large numbers (>2,000) of the parasites are collected to insure a broad genetic base.

Other Considerations in Production of Entomophages

Host production

In the United States, production of entomophagous arthropods to augment natural supplies is confined to a few private firms, cooperatives, and government agencies. But production and use of predators and parasites for control of arthropods is much more widespread worldwide. Nevertheless, without exception these entomophages are being mass-produced on live hosts or host products. The necessity of rearing the host and sometimes the host's food plant often more than doubles the cost and complexity of rearing the parasite or predator, restricting wider-scale usage of this technique in biological control.

In China, production of *Trichogramma* on oak silkworms mass-produced in the field on oak trees does circumvent many of the problems that come with producing host material in the laboratory insectary. And the oak silkworm cocoon can still be used for silk production. Others have extended this technique by producing the entomophages on hosts reared in the field. For example, Halfhill and Featherston (1973) reported a study where the parasite *Aphidius smithi* Sharma and Subba Rao was reared in field cages containing the host pea aphid, *Acyrtosiphon pisum* (Harris), and allowed to escape into surrounding alfalfa, *Medicago sativa* L., fields through temperature-controlled vents in the cage roofs. And Stevens et al. (1975) reported that the Mexican bean beetle, *Epilachna varivestis* Mulsant was controlled in soybeans, *Glycine Max* (L.), by release of the eulophid *Pediobius foveolatus* (Crawford) in nurse crops (snap beans, *Phaseolus vulgaris* L.) where they multiplied on Mexican bean beetle larvae and spread into adjacent soybean fields. Another technique for rearing entomophages in the field is the application of supplementary foods that induce oogenesis (Hagen and Hale 1974).

Artificial diets and in vitro rearing

Excellent artificial diets are available for rearing many phytophagous arthropods but are generally lacking for entomophagous arthropods (Singh 1977; see House 1977 for the most recent review of nutrition of entomophages). Development of suitable artificial diets could enable mass production of entomophagous arthropods for augmentative purposes at a cost competitive with other methods of insect pest control, and could insure production of an organism of consistent quality.

For predators, the most progress has been made in developing artificial diets for rearing *C. carnea*. Significant advances include development of an artificial diet for rearing larvae (Hagen and Tassan 1965; Vanderzant 1969, 1973), encapsulation of the larval diet (Martin et al. 1978), and development of a nutritious and practical adult diet (Hagen and Tassan 1970). Some progress has been made in artificial diets for rearing some of the coccinellids—for example, *Coleomegilla maculata* DeGeer (Atallah and Newson 1966).

The state of our knowledge on nutrition of parasitic Hymenoptera and Diptera is poor; however, some species have been reared in vitro. Bronskill and House (1957) reported limited success at rearing the ichneumonid endoparasite *Pimpla turionella* (Linnaeus) on pork liver. And Coppel et al. (1959) reported that pork liver was an excellent diet for rearing the sarcophagid *Agria housei* Shewell [= *Agria affinis* (Fallen) or *Pseudosarcophaga affinis* (Fallen)]. House (1954) developed a diet for rearing *A. housei* and continually refined it (House 1972). Bouletreau (1972) was successful at rearing the pupal endoparasite *Pteromalus puparum* Linnaeus in host hemolymph; and Hoffman and Ignoffo (1974) were able to rear this parasite on media containing yeast hydrolysate and bovine serum. Recently, Yazgan and House (1970) described a successful, chemically defined artificial diet for rearing the ichneumonid pupal endoparasite *Itopectis conquisitor* (Say). This diet was greatly improved by Yazgan (1972), so parasite mortality decreased; and House (1978) developed a method for encapsulating it. The hymenopterous ectoparasite *Exeristes roborator* (Fabricius) was also reared successfully on a chemically defined diet by Thompson (1975), and he continued these studies (Thompson 1977) to expand our knowledge on nutrition of hymenopterous larval parasites. Successes in rearing of the egg parasite *Trichogramma* spp. (Hoffman et al. 1975, Guan et al. 1978, Liu et al. 1979) show that a suitable artificial diet for rearing this insect is feasible. Likewise, recent successes in rearing tachinids in vitro (Grenier et al. 1975, 1978; Nettles et al. 1980) have expanded our knowledge on their nutrition. In fact, Nettles et al. (1980) demonstrated that the parasitic maggot

of *Eucelatoria bryani* Sabrosky does not have to attach itself to the host tracheal system to complete development.

Use of unnatural hosts

Many large-scale rearing programs have used unnatural hosts for production of entomophagous arthropods. Unnatural hosts are typically used in place of natural hosts because of costs, convenience, and ease of handling. In fact, four of the programs discussed above used unnatural hosts for mass production—*A. melinus* is reared on oleander scale fed squash, *C. carnea* is reared on Angoumois grain moth eggs from wheat-fed moths, *L. distraeae* is reared on greater wax moth larvae fed a cereal diet, and *Trichogramma* spp. are reared on Angoumois grain eggs from wheat-fed moths.

There has been some concern that entomophagous arthropods reared on unnatural hosts may change their host preference because of preimaginal conditioning and would therefore be less effective when released for control of the natural host (National Academy of Sciences 1969). It has been shown that rearing a parasite on an unnatural host increases a parasite's acceptance of that host. But Thorpe and Jones (1937) found no evidence that a parasite prefers the unnatural host to the natural after only a few generations. In fact, more recent research (Arthur 1965, Bryan et al. 1968, Legner and Thompson 1977) has failed to demonstrate this phenomenon. Selection for genotypes that respond more rapidly to unnatural host and survive better on it may explain some reported cases of preimaginal conditioning. In any event, parasites reared for a short time on an unnatural host will probably respond as strongly to the natural host as those reared on it.

Reduced vigor of entomophages because of exposure to laboratory hosts that supply inadequate nutrition to the attacker is probably the most critical problem in use of unnatural hosts for rearing (Morrison and King 1977). For example, *Trichogramma* spp. reared on unnatural hosts have been shown to have reduced size, fecundity, longevity, and general robustness (Lewis et al. 1976). And host diet has also been shown to affect the suitability of a host for parasite development. For example, Etienne (1974) reported that *L. diatraeae* could not be continuously reared on greater wax moth larvae fed beeswax and pollen, unless the host diet was supplemented with vitamin E or wheat germ. And vigor of *L. diatraeae* flies was improved when a cereal diet fed to greater wax moth larvae was supplemented with wheat germ and the protein content increased (King et al. 1979). Reduced vigor has also been demonstrated in parasites that have been reared on the natural host when it is fed different foods (Kajita 1973, Sato 1975, Altahtawy et al. 1976). So the

host's nutritive value to the parasite can be significantly modified by the food it eats. Finally, suitability cannot be determined merely by screening hosts, but host nutrition and other factors must also be considered; and compromises between entomophage quality and quantity may have to be made because of cost and the numbers required.

Storage

Most predators and parasites used in augmentation programs must be mass-produced as needed and released at a specific time in relation to development of the pest host. The ability to store the entomophage or host for long periods would greatly reduce costs and waste caused by necessary seasonal increases and decreases in production. Morrison and King (1977) reviewed techniques used in storing various species of entomophages and found that holding at low temperatures to reduce developmental rates is typically the major component in almost all reported storage techniques.

Quality control

Apparently, no one routinely measures traits or attributes such as genetic variation, diurnal rhythmicity, flight propensity, and flight ability to insure that essential behavioral characteristics are maintained. Most measurements now done assess production. These measurements include percentage of parasitization and of adult emergence, size or weight, sex ratio, and amount of egg production. These production measurements are, of course, essential for a consistent, physically standard product but do not relate directly to field performance.

Several researchers have divided quality into various components and then attached relative values to them based on the proposed use of the predator or parasite (see Boller 1972, Boller and Chambers 1976, Hoy 1976, and Huettel 1976). We have consolidated these into four components—adaptability, sexual activity, host selection, and motility. Increasingly, rearing programs require monitoring of traits that manifest these components; but few instances exist where these have been used to control quality in entomophagous arthropods (Huettel 1976).

Occurrence of the genetic bottleneck that insects go through during colonization is a well-established phenomenon (Boller 1972, Mackauer 1972). Production is usually low during a new colony's first few generations in the laboratory, but it increases after five to seven generations. Obviously, the colony is undergoing intense selection pressure for individuals having traits that enable them to best survive and reproduce under insectary conditions. But, because genetic material is often lost in the selection process, the organism's ability to survive and

reproduce in the field may be reduced. And genetic deterioration of the colony often becomes apparent only after it is irreversible, because behavioral traits are not routinely monitored. Periodically replacing the laboratory colony with field-collected material has partly solved this problem, but this is a myopic and costly solution. Excellent opportunities exist for implementing techniques to monitor and maintain essential characteristics and even select for desirable traits in mass-produced entomophages, but the necessary researchers have to be directly assigned these responsibilities.

Engineering

Engineering efforts such as development of flow charts combined with time-and-motion studies often lead to improved production and reduced costs. The trained engineer can often visualize existing production problems and their solutions better than an entomologist. So, a truly interdisciplinary approach involving engineers, entomologists, insect behaviorists, nutritionists, geneticists, and chemists is essential to production of entomophagous arthropods.

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A Laboratory Method for Mass Rearing the Eastern Spruce Budworm, *Choristoneura fumiferana*

By D. G. Grisdale¹

Introduction

The eastern spruce budworm, *Choristoneura fumiferana* (Clemens), is the most widely distributed forest insect pest in North America. Its range includes the Eastern States from Virginia to Minnesota and all of the forested regions of Canada from Newfoundland to Alberta, north-eastern British Columbia, the southern part of the Yukon Territory, and the southern half of the MacKenzie River basin of the Northwest Territories (Prebble 1975). It attacks balsam fir, *Abies balsamea* (L.) Mill.; alpine fir, *A. lasiocarpa* (Hook.) Nutt.; white spruce, *Picea glauca* (Moench) Voss; red spruce, *P. rubens* Sarg.; and black spruce, *P. mariana* (Mill.) B.S.P. At the start of eastern spruce budworm's life cycle in July and early August, moths deposit eggs in masses on host trees (Prebble 1975). The larvae hatch in about 10 days and spin silken hibernation shelters in crevices of bark, under bud scales or lichens, and in the cups of old staminate flowers. First larval molt occurs in late August in the hibernation shelter; the second-instar larvae remain there without feeding until the next spring. Overwintering larvae emerge in late April or early May and bore into old tree needles or into the unopened buds; or they feed on early opening staminate flowers when these are available. After a week or more, larvae move to opening vegetative buds and feed on the flaring needles under a protective silken shelter. Full-grown larvae pupate in the feeding webs in late June or early July. The moths emerge from the pupal cases about 2 weeks later, completing the annual one-generation cycle.

In the 1920's and 1930's, aerial insecticide dusting was used to control spruce budworms in small areas. In 1944, aerial spraying with chemical insecticides began, and such operations have been conducted in Canada every year since then (Prebble 1975). In that time, intensive research efforts have been made to develop more effective methods of managing spruce budworm populations. Before 1965, investigations were hampered by an inability to rear enough larvae for laboratory experimentation and propagation of pathogens such as viruses and microsporidia.

Stehr (1954) described a method for rearing spruce budworms throughout the year on shoots of balsam fir that had been preserved by freezing. But not until a synthetic diet was developed (McMorran 1965) was the rearing program able to expand. As the demand for budworm larvae increased, methods were developed for mass producing them (Grisdale 1970, 1972, 1973). This paper describes further modifications in rearing methods. Presently, at the Canadian Forestry Service's Forest Pest Management Institute in Sault Ste. Marie, Ontario, we are able to produce about 250,000 spruce budworm larvae weekly. We also mass-rear western spruce budworm, *Choristoneura occidentalis* Freeman, by the same method.

Colony Maintenance

Artificial diet

The artificial diet that we use (table 1) is like McMorran's (1965), though we substitute granulated agar for nutrient agar, use a veterinary grade of Aureomycin (chlortetracycline), and increase the amount of wheat germ. No For-

Table 1.—Composition of the spruce budworm diet and amounts of ingredients for about 3,600 ml of diet (mixed in 1-gallon blender)

Ingredient	Quantity
Granulated agar	60 g
Water (distilled), in blender	792 ml
Water (distilled), to dissolve agar	2,232 ml
Casein, vitamin free	126 g
4 M Potassium hydroxide	18 ml
Alphacel	18 g
Salt mixture, Wesson	36 g
Sucrose	126 g
Wheat germ	160 g
Choline chloride	3.6 g
Vitamin solution ¹	36 ml
Ascorbic acid	14.4 g
Formalin (37% formaldehyde)	1.8 ml
Methylparaben	5.4 g
Aureomycin powder (chlortetracycline hydrochloride, 55 mg/g)	20 g

¹100 ml contains 100.0 mg niacin, 100.0 mg calcium pantothenate, 50.0 mg riboflavin, 25.0 mg thiamine hydrochloride, 25.0 mg pyridoxin hydrochloride, 25.0 mg folic acid, 2.0 mg biotin, and 0.2 mg vitamin B-12.

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Figure 1.—Electric kettle (left) and vertical cutter mixer (right) used in preparing casein and wheat germ diet for rearing the eastern spruce budworm.



Figure 3.—Eastern spruce budworm larvae and gauze patch disposable plastic cup.

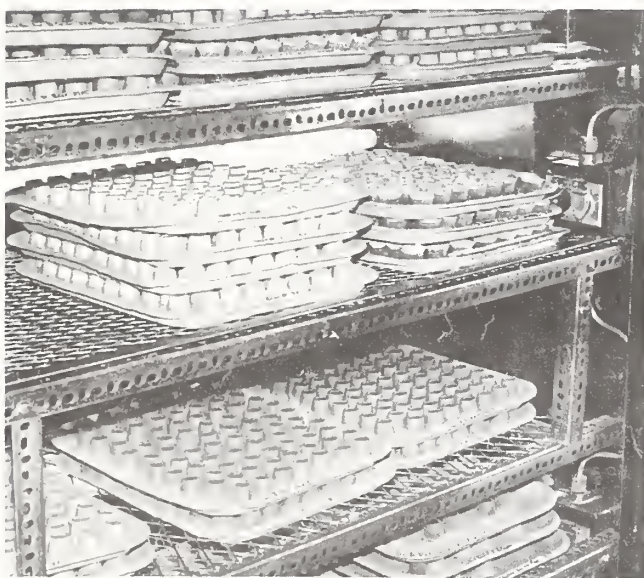


Figure 2.—Disposable plastic cups and trays used as rearing units for the eastern spruce budworm.

malin (formaldehyde) is added to diet used in the multiplication of virus because it may suppress the progress of virus infection in the host (Vail et al. 1968). The diet is prepared in 30-liter batches. An electrically heated steam-jacketed kettle (capacity 75 liters) is used to liquify the agar. The kettle (fig. 1) is fitted with a bridge-mounted anchor-shaped mixer with nylon scrapers driven by a 1-horsepower, variable-speed motor. Water is placed in the kettle. With the mixer operating at low speed, the agar is added and the heaters turned on.

While the agar is dissolving, water is added to a 40-quart vertical cutter-mixer, a Hobart VCM-40 with two speeds—1,750 r/min (revolutions per minute) and 3,500 r/min—and equipped with standard narrow knives and a hand-operated mixing baffle (fig. 1). The VCM is turned on at low speed and the remaining ingredients added in the order given in table 1. A large funnel, custom made to fit the largest opening in the inspection cover, is used for adding the ingredients. The funnel allows uninterrupted operation of the VCM and reduces splashing. The VCM is left on for about 1 minute after the last ingredient has been added, and the mixing action is supplemented by the hand-operated mixing baffle. During this primary mixing, some of the ingredients may stick to the underside of the cover; so the VCM is stopped, and this material is scraped off to insure that all ingredients

are in the diet and well mixed. The VCM is again operated at low speed and the liquified agar added through the funnel; the inspection cover is closed, and the VCM speed is increased to 3,500 r/min for about 3 minutes. The diet is now ready to pour.

About 15 ml of diet is hand-poured into 3/4-oz semitransparent ribbed plastic cups (Portion Packaging, Rexdale, Ontario). This container was selected because each second-instar larva can establish its own feeding site between the ribs and be relatively undisturbed by others in the cups until the fourth instar. Another advantage of these cups is that larval development can be seen directly through the cup, so the lid need not be removed. Disposable pressboard cafeteria trays (46 cm by 36 cm) are used to hold the cups. About 90 cups can be set up in each tray, which also serves as a unit for rearing the insects (fig. 2). Trays are light and inexpensive and withstand autoclaving once or twice before being discarded. After the diet has cooled, it is sprayed with an antifungal solution that is a modification of the one described by Chawla et al. (1967). It contains 1.5 g sorbic acid and 0.6 g methylparaben (methyl *p*-hydroxybenzoate) in 100 ml of 95% ethyl alcohol. The alcohol solvent evaporates rapidly, leaving a thin film of antifungal agents on all exposed surfaces inside the cup. Trays of prepared diet are placed in plastic bags (six trays per bag) that are sealed and refrigerated at 0°–1° C until ready for use.

Larvae

Second-instar larvae in hibernacula and with diapause requirements satisfied are removed from cold storage and held at 20°±1° C for 24 hours. Then they are placed in a lighted rearing cabinet at 22°±1° C and 70% relative humidity. After 1 additional day, or before larvae emerge from hibernacula, a cheesecloth-Parafilm roll is cut into strips on scored lines. These strips are cut into appropriately sized patches (with 25–40 larvae each) and placed in diet-filled cups. These are then capped with lids made of unwaxed paper. To reduce the incidence of fungus infection, the cups are inverted and tapped to insure that the gauze patch (fig. 3) falls onto the lid and away from the surface of the diet.

When a specific number of larvae per cup is required, a large gauze patch is placed in a glass dish (19 cm in diameter) sealed with Parafilm. After emergence, larvae are transferred individually to the diet by a camel's-hair brush. The dish must be moistened often; if emerging larvae are held overnight, the dish should be kept in a cool (19°±1° C) darkened area to reduce larval activity and mortality. Emergence time of second-instar larvae may be shortened by at least 24 hours if patches are held in constant light at 24°±1° C and kept moist and the container is opened often for airing.

Colonized cups are exposed to 18 hours of light, temperature of 24°±1° C, and relative humidity of 60% until the larvae reach the fourth or fifth instar. This is the point in the Institute's program when most spruce budworms are distributed for testing and virus multiplication. Larvae to be used for the maintenance of rearing stock or for physiological studies are transferred to cups (six or seven larvae each) containing 10 ml of diet. If the cups are allowed to remain crowded, cannibalism becomes excessive and pupal and adult sizes are sharply reduced. Larvae are easily sexed; so, for special requirements, they can be reared separately from this time. (Paired testes of male larvae are readily visible in third and later larval instars.) When larvae are in the sixth instar and near pupation, trays of cups are inverted so that lids are upright. Because most larvae pupate near the lid, pupae are more easily harvested with the cups in this position. Larvae spend about 4 days in each of the second, third, and fourth instars, and 5 days in the fifth. Male larvae require about 8 days in the sixth instar before pupating; females need 2 or 3 more days.

Diapause-free strains of spruce budworm may be developed as a standard laboratory culture. A few non-diapause larvae (commonly called wandering seconds) are often observed in families produced from field-collected material. Shortly after molting into the second-instar, these larvae leave the hibernacula. Selection over six generations may yield a strain that is nearly free from diapause. (See Harvey 1957 for a description of diapause-free development and of rearing conditions necessary to produce a reliable laboratory strain.) But, because of constantly fluctuating research demands, we have found that removing second-instar larvae from cold storage when and if required serves our needs more efficiently than developing diapause-free strains.

Pupae

After pupation, pupae are sexed by the method of Jennings and Houseweart (1978). Because we sex so many pupae, we find that the best method for general rearing purposes is to count the abdominal segments visible ventrally and posteriorly to the wing pads (females have three segments; males have four). Experienced personnel observing these characteristics and the general shape of the pupae rarely err. For studies requiring segregated virgin moths, sexing by the location and shape of the genital opening may be used. Sexed pupae are placed in well-ventilated plastic crisper trays (27 by 20 by 10 cm) for adult emergence (fig. 4). To facilitate eclosion, the bottom of the tray is carefully lined with paper toweling fastened with tape to prevent the liner from shifting when adults are removed. To synchronize adult emergence, male pupae are held at a temperature 2° C lower than female pupae. The pupal stage lasts about 8 days.



Figure 4.—Sexed eastern spruce budworm pupae and emerged adults in ventilated plastic crisper trays.



Figure 5.—Eastern spruce budworm mating and oviposition cage. Note plastic shield on cage door.

Mating and oviposition

A screened cage (35 by 35 by 25 cm) is used for mating. The cage (fig. 5) has a sliding glass door; a paper towel covers the bottom. Four or five small balsam fir branches about 35 cm long are placed on the bottom of the cage as oviposition sites. This amount of foliage seems to reduce the disturbance of females when they are laying eggs and results in larger and more uniform egg masses. Normally, 200 pairs of adults are transferred to a cage (after a short

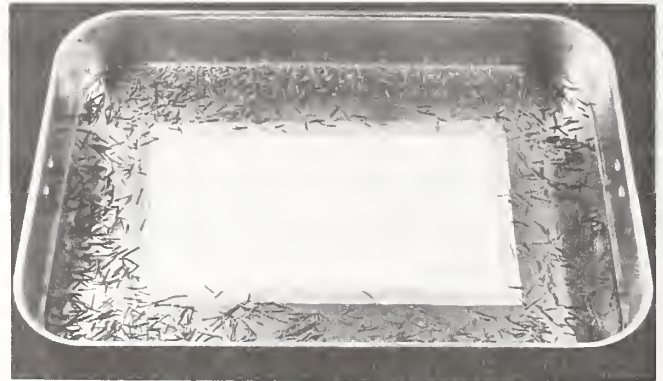


Figure 6.—Eastern spruce budworm eggs in black-headed stage attached to balsam fir foliage distributed evenly near gauze patch on aluminum pan bottom.

time in a refrigerator) in a 12- by 15-mm shell vial. A movable plastic shield is placed behind the sliding cage door and is most helpful in preventing adults from escaping when they are first placed in the cage and later when branches are removed. Up to 300 pairs of adults may be introduced into cages with good results. Tests showed that 150 pairs produced 28,653 eggs, or an average of 191.0 eggs per female; 200 pairs produced 41,293 eggs, or 206.5 eggs per female; 250 pairs produced 52,507 eggs, or 210.0 eggs per female; and 300 pairs produced 61,025 eggs, or 203.4 eggs per female (C. B. Budar and D. G. Grisdale, unpublished data). Adults are held at $20^{\circ} \pm 1^{\circ} \text{C}$ and relative humidity of 80%–90% with 12 hours of light. Cages are sprayed generously with distilled water two or three times daily. Egg laying is largely completed about a week after mating. Adults are killed in an oven and discarded on the eighth or ninth day after introduction into cages.

Eggs

Branches with egg clusters attached on needles are removed from the mating cages every second day. To prevent excessive adult movement, cages are sprayed with water just before branches are changed. Needles bearing egg clusters are removed from branches by surgical scissors or forceps. To reduce the amount of foliage material and for a better estimate of how many eggs are placed in hatching pans, the needles are cut just below the egg cluster. Eggs are placed on uncovered disposable cardboard trays and exposed to 18 hours illumination, temperature of $24^{\circ} \pm 1^{\circ} \text{C}$, and relative humidity of 55%–60% until they reach the black-headed stage and are about to hatch. Holding egg masses of the same age until they reach this stage insures uniform egg hatching and



Figure 7.—Sealed pan with attached cheesecloth on Parafilm for hatching and establishment by eastern spruce budworm second-instar larvae.

larval spinning and shortens the time larvae spend in the hatching and spinning unit. Also, the water content of the needles is reduced and practically eliminates any problems with fungus. Larvae hatch about 8 days after oviposition.

Hatching and spinning

An aluminum roasting pan (Supreme Aluminum of Canada, No. 4910) is used as the hatching and spinning unit. This pan is particularly well suited because of its convenient size (45 by 30 by 6.5 cm). Its rounded edges permit a perfect seal with Parafilm; it is easily cleaned and sterilized; and unlike glass or plastic, it is extremely durable. (Smaller pans of the same type are used when there are not enough eggs for efficient use of the larger pan.) A double layer of cheesecloth (30 by 15 cm) is attached to a slightly larger piece of Parafilm, which is then pressed into the bottom of the pan. Egg masses in the black-headed stage (enough to produce about 16,000 second-instar larvae) are distributed evenly over the bottom of the pan but away from the gauze (fig. 6). The pan is then tightly sealed with Parafilm. On the underside of the Parafilm is a patch (40 by 25 cm) of cheesecloth. The Parafilm is attached to the pan with strips of masking tape pulled downward and away from the rounded edge to insure an escapeproof seal (fig. 7). Care should be taken during the sealing operation to prevent the pan from tilting so that eggs do not shift from their original position.

Stocks of cheesecloth on Parafilm of various sizes are prepared in advance. Cloth is readily attached to Parafilm by rolling firmly with a cardboard roller (such as the Parafilm core). The cheesecloth is next scored (to reduce

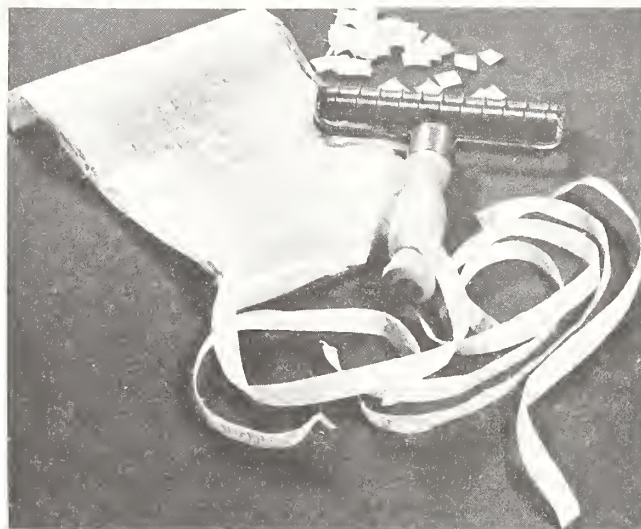


Figure 8.—An opened cheesecloth-Parafilm roll, strips and patches of second-instar eastern spruce budworm larvae, and the roller used in the preparation of strips.

larval deaths when Parafilm is cut with scissors at the time of patching) with a brass roller (fig. 8). The roller was made on a lathe from a piece of brass stock; it is 14 cm long, and its rounded circumferential ridges are 1.4 cm apart and 1.5 cm high. The scoring is done on a surface covered with heavy paper to prevent perforation of the Parafilm by the roller. When it is rolled with very firm pressure, the roller's ridges score the surface of the cheesecloth and embed the fibers into the Parafilm. The first-instar larvae cannot spin hibernacula in these scored areas, so the spun-up larvae appear as regular strips on the cheesecloth.

Hatching pans are held at a temperature of $22^{\circ}\pm 1^{\circ}$ C under continuous lighting. Once large numbers of first-instar larvae are observed crawling on the upper gauze, the pan may be turned over and rotated periodically during spinning. This rotation insures a uniform distribution of larvae throughout both pieces of gauze. Silk laid down by hatching larvae cements needles to the bottom of the pan where they remain firmly attached even when the pan is turned over. As soon as second-instar larvae are observed in hibernacula, the sheets of Parafilm with attached gauze are removed, rolled loosely, sealed individually in small plastic bags, and placed in a darkened area at an incubator temperature of $19^{\circ}\pm 1^{\circ}$ C. Larvae are held at this temperature for about 3 weeks from the date of hatching and are then ready for cold storage. This prestorage treatment of second-instar larvae is a critical part of the rearing technique because it results in

significantly higher storage survival (McMorran 1973). (See Harvey 1957 for a description of the behavior pattern of the laboratory-reared spruce budworm from hatch to building a hibernaculum where it molts and enters diapause.)

Hibernation

Plastic bags of young larvae in hibernacula are placed in appropriately sized brown paper bags to facilitate recording and shelf storage. Insects are placed in a coldroom, at a temperature of 1° C, where they remain for 18–35 weeks. Moisture is not added to the plastic bags before storage, because it may promote the growth of fungus.

Production Management

Trouble-free budworm rearing depends on several factors. Probably the most important is having dedicated, competent rearing personnel. Strict adherence to rearing techniques, sanitation practices, quarantine regulations, and the use of rearing units with adequate environmental control are also important factors. As anyone involved in insect rearing knows, hardly a day goes by without a minor problem, and occasionally major ones occur. Problems happen most typically with artificial diet, fungal infection, and worker health and safety.

Artificial diet

Artificial diet of consistently good quality is an important factor influencing the quality of laboratory-reared spruce budworms. At first, diet was prepared in an electrically heated kettle fitted with a bridge-mounted mixer and dispensed into cups by an automatic filler (Grisdale 1973). Diet prepared in this manner was of good quality and met our requirements, but comparative tests of larval development showed that larvae reared on diet prepared in smaller, more easily mixed amounts in a 1-gallon blender consistently developed at a faster rate. And occasionally a batch of diet prepared in the kettle produced budworms that developed slowly and irregularly; when many thousands of larvae were involved, production schedules were disrupted. Inadequate mixing and the length of time diet remained in the kettle while being dispensed were primary suspects. So the Hobard VCM was put into operation in 1976. Since that time, all prepared diet has consistently been of high quality except in a few instances when the ingredients (wheat germ in one case, salt mix in another) were of poor quality. But, in both cases, we were able to trace the source of defective ingredients quickly by examining records on diet preparation.

Records are kept of the dates when new units of diet ingredients are first opened, of the temperature of the liq-

uified agar, of the diet temperature when it is dispensed, and of other pertinent information on each lot of diet. Diet ingredients are purchased from firms that have provided us with quality products in the past. Most diet ingredients are refrigerated as recommended by the supplier; wheat germ that will not be used within 3 weeks is stored in a freezer. We have tried to lower cost of producing diet during times of austerity, but we have found that less expensive products are often of unreliable quality and not suitable for budworm rearings. Of the several insect species that can be reared on the same lot of diet (Grisdale 1973), only spruce budworm is noticeably affected by poor diet.

Control of fungi

Fungal development on synthetic diets and infection of second-instar larvae both before and after storage can create serious rearing problems unless careful sanitation practices and rearing techniques are closely followed. Other infection sources are present in our laboratory because we rear as many as 12 different insect species at any one time in the same rearing facilities. Some species—such as silkworm, *Bombyx mori* (Linnaeus); hemlock looper, *Lambdina fiscellaria fiscellaria* (Guenée); and the oleander hawk moth, *Deilephila nerii* (Linnaeus)—are reared on foliage; if trays are not cleaned frequently, fungus develops on frass and uneaten leaves. Handling of all such fungus-infected material is carried out in a biological containment hood. Other measures to combat fungi are use of antifungal agents in the diet, surface spraying, and environmental controls. The unwaxed cup lid significantly reduces fungus contamination. But the inverted cups should not be incubated on a smooth surface, which stops air transfer within the cup and so permits the incidence of fungus infection to increase greatly.

Since balsam fir foliage used as an oviposition site is considered a principal source of infection, branches are placed in plastic bags in a 1% solution of household bleach (Javex, 6% available sodium hypochlorite), agitated until the foliage is thoroughly wet, and then refrigerated for use as required during 1 week. Other steps for preventing fungal contamination are removing young larvae from hatching pans shortly after hatch, keeping prestorage treatment of second-instar larvae brief, and storing larvae without adding moisture. Following these procedures should eliminate fungal infection from budworm rearings.

Worker protection

Worker protection during the production of spruce budworm has received considerable attention in recent years, particularly since transient allergic responses do occur

and can sometimes be incapacitating. At the Institute, the problem is further complicated because we rear several species and because we produce large numbers of insects such as *Orgyia* spp. that have both wing scales and urticating larval hairs. Workers were required to wear protective face masks and gloves when handling adults or obnoxious materials. Work was carried out under a fumehood; but, because our building design did not allow adequate venting, fumehood efficiency was reduced. And the allergens continued to cause problems despite these precautions. The hazard was severe enough to justify the deletion of some species from rearing even though considerable financial investment was involved. In a further effort to reduce the problem with scales, we installed a biological containment hood in the main rearing room. The hood, designed for worker protection only, proved very effective in protecting staff from obnoxious materials. Two more hoods were installed in other areas of high contamination in the building, and the health hazard has been reduced to a very low level.

Quality Control

We have not yet greatly emphasized inline testing for insect quality except for regular sampling of laboratory adults for the microsporidian parasite *Nosema fumiferanae* (Thomson). Also, efforts are made to obtain disease-free field insects for yearly introduction into laboratory rearing stock. The principal requirement at the Institute has been for a vigorous larva free of microsporidian spores for general experimentation and propagation of pathogens. Even with the microsporidian present, the use of an antifungal agent, benomyl,² allowed the production of a standardized larva.

Rearing stocks that are free of microsporidian infection

One of the most important factors limiting production of healthy spruce budworm larvae is the microsporidian parasite *N. fumiferanae*. In the past, as production of budworm larvae increased, we experienced a recurring infection of laboratory stocks by this pathogen. Wilson (1980) describes *N. fumiferanae* as a debilitating agent, under natural conditions, that affects host vigor, longevity, and fecundity. Rearing larvae on synthetic diet in the laboratory appears to mitigate the effects on host vigor and longevity; but fecundity is reduced. And, unless measures are taken to reduce levels of infection, larvae are unsuitable for research purposes because the presence of the parasite interferes with interpretation of experiments

involving other pathogens. Also, in virus propagation programs, yields are greatly reduced.

Microsporidia are widespread in nature, affecting all larval instars, pupae, and adults of the spruce budworm (Nielson 1963). Wilson (1973) found that there is a general buildup in the *N. fumiferanae* infection as the budworm infestation ages over 2 or more years. It had been our practice to collect field pupae each year from areas of new budworm infestations where the incidence of parasitism and disease was generally low (Grisdale 1970). Mating and handling of all field stock was done individually. Then both male and female adults from successful matings were examined microscopically for spores. Progeny of moths free of disease were kept for laboratory rearing stock; infected progeny were discarded or used in virus-multiplication programs. The field stocks considered free of disease were crossed with existing laboratory stock to increase vigor and maintain genetic variability. Because we handled so many insects, we made no further attempt to diagnose adults from multiple matings. In spite of our precautions, we were never able to completely eliminate microsporidia from our rearings, probably because spores in microscopically inspected adults may be overlooked even after two generations of individual rearings (G. G. Wilson, personal communication). A few missed spores can quickly build up, particularly under mass-rearing conditions. In the laboratory, spores in frass and regurgitate are infectious to healthy larvae. And Wilson (1972) found that infected female but not male adults readily transmit *N. fumiferanae* to offspring.

So, in 1978 we began a determined and successful effort to eliminate microsporidia from our rearings. Field-collected insects from several Ontario infestations were reared in a room separated physically from the main rearing room. The procedures we used for individual mating, spinning, and storage were those described by McMorran (1965). Progeny of adults diagnosed as free of infection after two generations of controlled rearings were introduced to the general rearing operation. Small, representative samples from each geographical location were held for one more generation of controlled rearing. We began sampling of adults from all multiple matings. Cage number and origin of stock are recorded; progeny are assigned this identification; and, after diagnosis, the presence or absence of microsporidian spores is recorded. Because as many as 20,000 adults may be processed weekly, only a representative sample of 50 adults per cage can be conveniently examined.

Rearing stocks with microsporidian infection

Each year, several million larvae of acceptable quality and enough overwintering larvae to implement these rearings

²Methyl 1-(butylcarbomoyl)-2-benzimidazolecarbamate as Benlate, a wettable powder with 50% available benomyl.

have been produced from stocks infected with *N. fumiferanae*. Benomyl has been used routinely in this laboratory since 1975 to reduce the incidence of microsporidian infection in larval rearings. At 100 p/m (parts per million) incorporated into the synthetic diet at time of preparation, benomyl effectively limits microsporidian multiplication during the period budworm larvae are exposed to it. Larvae so treated are satisfactory for most experimentation and for virus-multiplication programs. And, because benomyl is such an effective fungicide, diet in cups rarely becomes contaminated. Of course, larvae reared on diet containing benomyl are not suitable for studies involving microsporidian parasites or fungi. Larvae reared on this treated diet have reduced mating success, so they should not be used for stock maintenance.

Also Harvey and Gaudet (1977) discussing the effects of benomyl in varying concentrations, showed that it effectively limits microsporidian infection in spruce budworm larval stages; but its effect does not carry over into the adult stage. Microsporidia multiply vigorously at the end of the sixth instar and during the pupal stage. Benomyl in concentrations of 75 p/m and above reduced budworm growth and fertility. The most notable effect was the reduction in fertile matings and in percentage of eclosion from eggs. Males were more sensitive than females to benomyl.

With these findings in mind, we rear infected budworms to be used for stock maintenance on benomyl diet until they reach the late stages of the fifth instar. At that time, the number of larvae per cup is reduced to six, and they are placed on diet containing no benomyl. Feeding on the benomyl-free diet throughout the last instar diminishes some of benomyl's undesirable effects. And, though microsporidian infection is still present, egg production and egg hatch appear near normal. Handling of insects during research, which is sometimes done in less than ideal conditions, may result in fungus contamination. So we are often asked to rear even disease-free insects on the benomyl diet.

Field tests of adults reared in the laboratory

Field trials using laboratory adults have been few. And we have not been asked to add to this sparse information on the differences between laboratory and wild insects. Testing to date has shown little apparent difference between laboratory and wild females in their ability to attract wild males under field conditions (C. J. Sanders, personal communication). But Ennis and Charlebois (1979) reported that field tests showed that some inbred laboratory strains do not perform well in the field. Laboratory-reared males from our randomly outbred stock, however, responded like field males to pheromone

traps—released males were recaptured after 3 days even when field populations were very high. And, though recovery in pheromone traps of released males from an orange-eyed mutant strain (Ennis 1978) that had been inbred for 6 years was very poor, it was near normal for orange-eyed males previously crossed with field stock to introduce genetic background from wild insects (Ennis and Charlebois 1979).

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Fractional Colony Propagation

A New Insect-Rearing System

By J. David Hoffman, C. M. Ignoffo, Paula Peters,¹ and W. A. Dickerson²

Introduction

Improvements in performance of insect colonies result mainly from advances in nutrition, rearing facilities, procedures, equipment, and environmental control. But little emphasis has been placed on one of the most important factors in insect rearing—selection to improve performance of the colonized insect. Fractional colony propagation is an insect-rearing concept that involves separate colonization of many subcolonies started from one parent colony. Its objectives are to increase management control and monitoring of the colonies, increase their productivity, reduce extremes in population fluctuations, and control spread of pathogens and microbial contaminants.

Fractional Colony Propagation

The system using fractional colony propagation was first applied in 1978 to a colony of cabbage loopers, *Trichoplusia ni* (Hübner), that produced 500 pupae per day. A new subcolony of *T. ni* was established each day for 26 days (26 subcolonies). Each subcolony was numbered consecutively and reared separately from other subcolonies. To maintain the genetic diversity of the subcolonies, eggs were obtained from the parent colony on different days. A rating system, based on one major criterion (fecundity) and five secondary criteria (hatch, larval development, pupation, emergence, and disease), was used to evaluate each subcolony.

All egg sheets were visually rated as either high (80 eggs/6.5 cm²), medium (50 eggs/6.5 cm²), or low (20 eggs/6.5 cm²). These densities were based on the fecundity of the parent colony during the 12 months before subcolonization. The number of eggs on sheets accumulated for each subcolony in a generation was summed to estimate total fecundity.

Hatch, larval development, pupation, and emergence were monitored on specific days to evaluate the performance of

the subcolony. Disease and microbial contamination in the subcolonies were monitored continually. Eggs from each subcolony were collected and refrigerated (at 12° C, about 6 days) until percentage of hatch could be estimated. An egg hatch of more than 90% (based on a sample from each egg sheet) was acceptable. Rate of larval development was evaluated on the sixth day after egg hatch because this stage can be visually estimated more rapidly and accurately than earlier stages of larval development. At least 80% of the larvae reared 6 days at 30° C should be in the fourth instar. Rate of pupation was determined on the 11th day after egg hatch. At least 90% of the population reared at 30° C should have pupated by this time. A count of dead and living pupae 19 days after egg hatch was used to determine percentage of adult emergence. Adult emergence of less than 90% was considered abnormal. Actual measurements of the first four secondary criteria were recorded only when a visually estimated value fell below the expected performance level. Subcolonies rated for two consecutive generations below the expected performance level for hatch, larval development, pupation, or emergence were replaced with higher-rated subcolonies.

A subcolony with any sign of disease was immediately discarded and replaced with eggs from a subcolony as far removed in time as possible from the diseased subcolony (for example, refrigerated eggs collected 1 week earlier). As a precautionary measure, any subcolony showing questionable signs of disease (abnormal growth because of nutrition, microbial contaminants, or environmental factors) was also replaced with better subcolonies.

Converting the single-colony propagation system for *T. ni* to fractional colony propagation did not require additional labor, space, or facilities; nor did it increase rearing complexity. The workflow for both systems was essentially identical except that a subcolony was started each day, and progeny from different subcolonies were never mixed (table 1).

Effects of the System

The system using colony propagation greatly improved productivity and stability of the *T. ni* colony. And the subcolony system allowed monitoring and manipulating of the colony from generation to generation. Such management control was not possible with the single-colony rearing system. So the productivity of the colony

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Table 1.—Comparative flow of work for single- and fractional-colony propagation of *Trichoplusia ni* (Hübner)

Day	Work common to both rearing systems	Work specific to fractional colony propagation
0	Place eggs with diet; mark with date set up	Mark with subcolony number; never mix eggs from different subcolonies.
8	Estimate percentage of 4th-instar larvae (6 days after hatch).	Count larvae if estimate for 4th instar is less than 80%; record value if below 80%.
13	Harvest pupae; estimate percentage of pupation.	Count pupae if estimate for pupation is less than 90%; record value if below 90%; never mix pupae from different subcolonies.
14	Surface-sterilize pupae	
15	Place pupae in emergence-oviposition cage and date.	Mark with subcolony number.
18	Initial emergence (females); feed 10% honey solution daily.	Never mix moths from different subcolonies.
21	Estimate percentage of emergence based on number of live and dead pupae; collect 1st egg sheet; date egg sheet; estimate egg density—high, medium, low; mark egg-density estimate on egg sheet; record egg-density rating for each egg sheet; surface-sterilize egg sheet.	Count pupae if estimate for emergence is less than 90%; mark egg sheet with subcolony number; never allow egg sheets from different subcolonies to mix.
22	Cut egg sample from egg sheet and incubate at 30° C; refrigerate egg sheet at 12° C.	
26	Stop collecting egg sheets from each cage when egg sheet is rated low; estimate percentage of hatch of egg sample, and record.	Determine percentage of hatch by counting a sample of eggs if estimate of hatch in egg sample is less than 90%; sum all egg sheets to estimate total fecundity, and record.

(based on fecundity) increased more than 30% above the mean within six generations (subcolony selection began at the third generation). Higher fecundity may possibly be gained with more selection.

Production stability, measured as fluctuations in fecundity, improved rapidly. Before the colony was subcolonized, average fecundity fluctuated unpredictably (about 30%). After subcolonization, fluctuation in the average number of eggs was reduced sixfold within six generations.

Pathogens introduced at low levels usually spread throughout an entire colony before they are detected and often cause lower productivity, slower rates of development, and reduced egg hatch. So, subcolonies with these conditions can be replaced, even before the disease symp-

toms appear. Fractional colonization minimizes contact between subcolonies; so it should reduce the spread of a pathogen. Subcolonies that are contaminated are easily replaced with another subcolony.

Subcolonies will probably become more homogeneous by continuous inbreeding. But pooling of progeny (eggs, larvae, pupae, or adults) from each subcolony for laboratory or field experiments should help maintain heterogeneity.

Although the full impact of fractional colony propagation has not yet been fully realized, the increases in management control, productivity, and production stability were excellent. This system should be applicable to both small and large rearing programs with two or more setups per generation period.

Production of Insects for Industry

The Dow Chemical Rearing Program

By W. R. Fisher¹

Introduction

In the mid-1970's, Dow Chemical U.S.A. recognized that its research program for insecticide development would be improved by increasing the uniformity and quality of the test insects. The company decided to construct a new rearing facility at its agricultural chemical research center in Walnut Creek, Calif. This paper describes the development of their new program designed for rearing the western spotted cucumber beetle, *Diabrotica undecimpunctata undecimpunctata* Mannerheim, on natural corn diet and for rearing four lepidopteran species on artificial diet: codling moth, *Laspeyresia pomonella* (Linnaeus); tobacco budworm, *Heliothis virescens* (Fabricius); beet armyworm, *Spodoptera exigua* (Hübner); and black cutworm, *Agrotis ipsilon* (Hüfnagel).

The previous program had been beset by problems that were impractical to solve in the existing facility. Microbial contamination was the most critical problem. Disease destroyed beet armyworm colonies and significantly reduced yields of tobacco budworm. The spread of disease among colonies was always a threat in the small holding rooms that contained more than two species. A colony devastated by disease had to be reestablished from clean stock, a process that took a month or more. Sublethal infections reduced colony vigor and resulted in test organisms that were poor in quality and abnormally susceptible to chemical challenge. Contaminants, such as mold, thrived on the artificial diets used to rear tobacco budworm and codling moth. The fungi covered much of the diet surface, competed with larvae for nutrients, and caused relative humidity in rearing containers to be above optimal levels for the insects, thereby reducing their yields and quality.

All microbial contaminants significantly increased the time, labor, and money needed to satisfy colony and research requirements. Contamination was difficult to

control in the old facility for several reasons. Most importantly, artificial diet was prepared in an area that was not adequately isolated from larval holding rooms. In fact, containers with pupae from these rooms were taken through this area to be harvested, exposing freshly poured diet to contaminants. Inadequate filtration of conditioned air, unrestricted movement of employees and other persons, poor sanitary procedures, and an inability to sterilize reusable containers also contributed to the problem.

Health hazards, inherent in any rearing program, were not adequately controlled in the old facility. For example, fungi growing on artificial diets produced potentially infectious spores that were inhaled by workers harvesting pupae or washing containers. Wing scales from lepidopteran oviposition cages and airborne dusts generated during mixing of artificial diets caused allergic symptoms in some employees.

Finally, procedures for rearing certain species were not compatible with an efficient and sanitary program. For example, codling moth larvae were reared in plastic containers covered by loose-fitting glass lids. The plastic was heat labile and could not be sterilized in a steam autoclave. The lids allowed late-instar larvae to escape into the holding room where they would pupate in walls and shelves, making sanitation difficult. Furthermore, to reduce desiccation, the surface of the artificial diet used for the codling moth larvae was coated with melted paraffin. But, in the process, wax would build up on the inside of the square-cornered containers and make sterilization by immersion in sodium hypochlorite impossible. So mold was common, and yields were low, thus necessitating more containers and labor to satisfy research needs.

These experiences with the previous program provided a basis for development of the new rearing facility. Five major factors were considered during its design: isolation of critical work areas, the flow of materials and products, room characteristics, equipment, and regulations and procedures.

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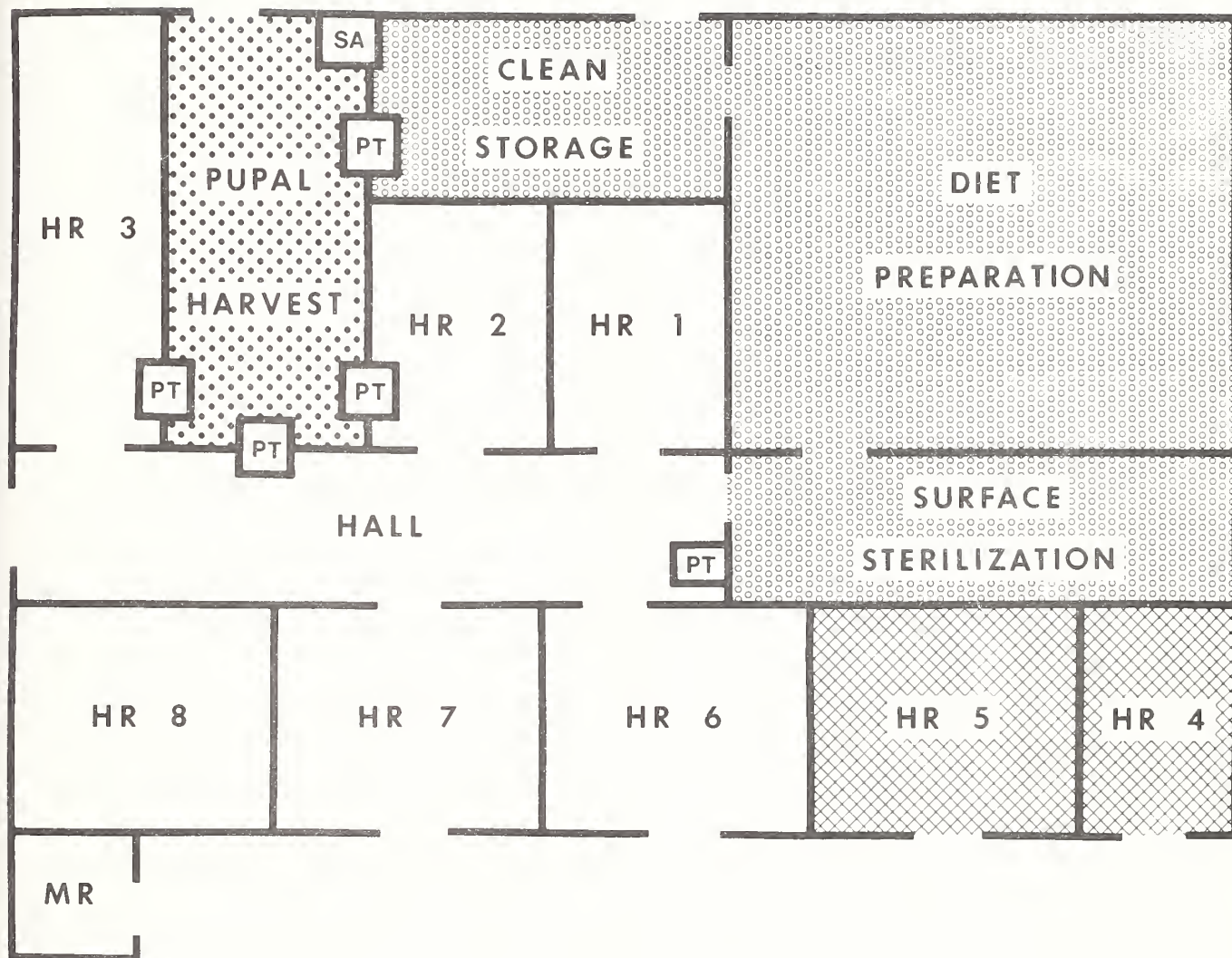


Figure 1.—Isolation of critical work areas in the Dow rearing facility. Open circles—clean work area. Solid circles—dirty work area. Cross-hatching—isolated room for holding insects. PT—passthrough. SA—steam autoclave. HR—insect holding room. MR—mechanical room containing motor-control center, water heater, boiler, telephone panel.

Development of the Dow Rearing Facility

Isolation of critical work areas

Isolation to maintain cleanliness in a rearing facility requires separation of operations, products, and materials. It also requires establishment of barriers to prevent spread of contaminants throughout the building. To get such isolation, the Dow facility was divided into three major sections. The first and most critical, the clean work area, consists of clean-storage, diet-preparation, and

surface-sterilization rooms (fig. 1). Only uncontaminated materials and insect eggs and pupae sealed in clean containers are allowed into this area. Sterilized, reusable containers and cages and other materials are kept in the clean-storage room. In the diet-preparation room, dietary ingredients are weighed and mixed, and diets are dispensed into containers and planted with eggs. Also in this room, surface-sterilized pupae are distributed into oviposition cages. Insect eggs and pupae are surface-sterilized with sodium hypochlorite in the room next to the diet-preparation room.

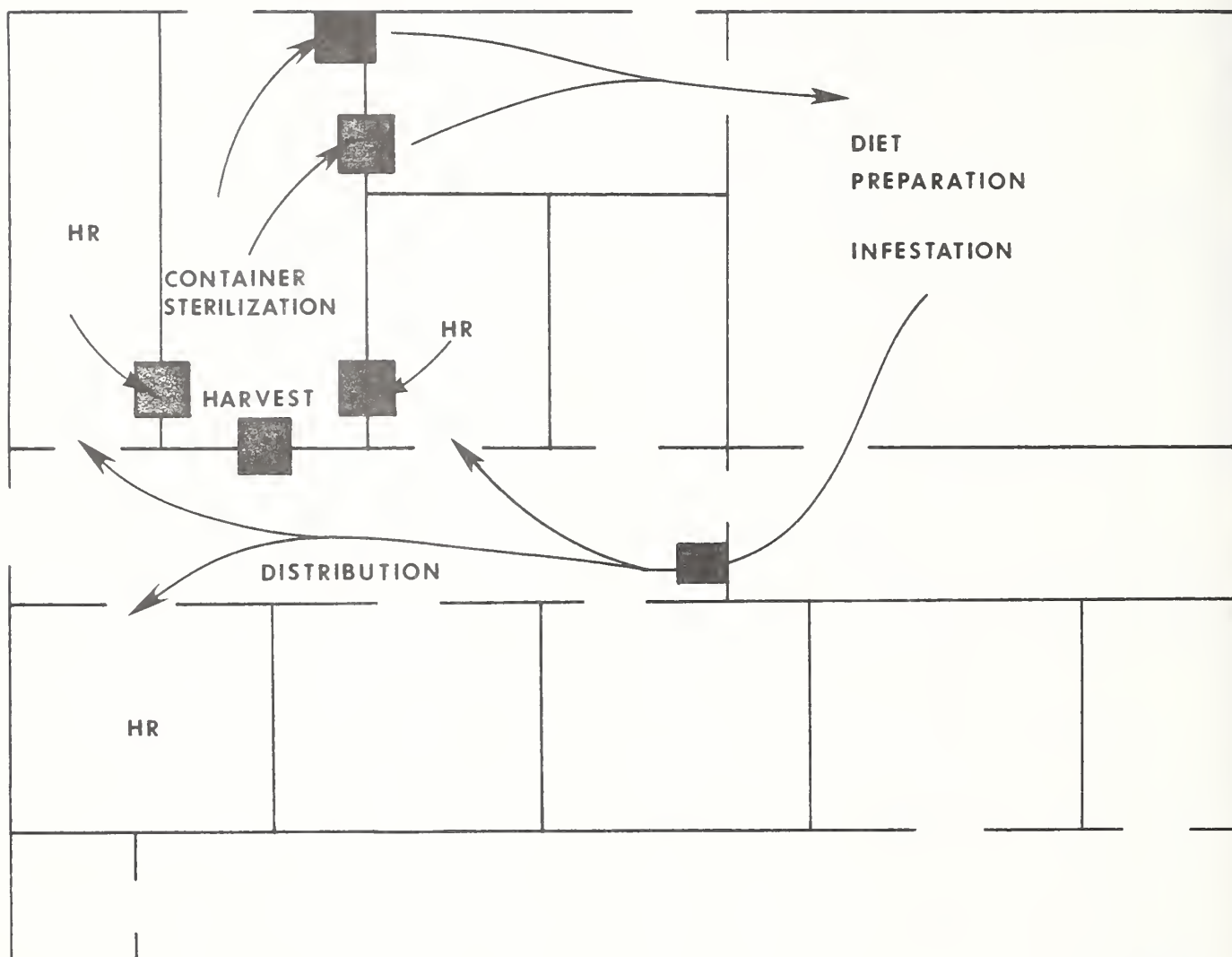


Figure 2.—One-way flow plan for materials and products in the Dow rearing facility.

The next level of isolation consists of eight holding rooms where insects are kept during larval development and adult oviposition. Rooms 4 and 5, the most isolated, are used to maintain parasites or predators for studies of their susceptibility to insecticides. This area also serves to isolate potentially diseased insects collected from wild populations before they are infused into production colonies. Rooms 1–3 and 8 are used to hold adults and developing larvae reared on artificial diets. The western spotted cucumber beetle, the only species reared on natural diet, is maintained in holding rooms 6 and 7.

The final area of isolation is the room for pupal harvest and container sterilization. Because the operations here involve dirty containers, a high potential exists for the

release of contaminants into the rest of the facility. So four ultraviolet-light passthroughs and a double-door, steam autoclave were installed to allow movement of products and materials in and out of this room without spreading contaminants. All tasks that liberate contaminants, such as opening larval containers and harvesting pupae, are done inside a fume hood.

Flow plan of materials and products

The flow plan (fig. 2) provides a logical, one-way movement of materials and products that links isolation areas in the facility. Containers from clean storage are taken to the diet-preparation room, filled with diet, and planted with eggs. They are then distributed to holding rooms

where they remain until completion of larval development. Next, containers are passed to the harvest room, where pupae are removed from the diet. Reusable containers are washed, sterilized, and transferred into the clean-storage room. Pupae are placed in clean cups with tight-fitting lids and taken to the surface-sterilization room where they are washed in sodium hypochlorite, placed in cages, and taken to the appropriate holding room for adult eclosion and oviposition. Sheets containing eggs are collected from these cages, placed in clean plastic bags, and taken to the surface-sterilization room. Rooms 2 and 3 are next to the pupal harvest area and hold larval stages that are susceptible to pathogens and that have the greatest potential for facility contamination. Direct access to the harvest room via passthroughs with germicidal lamps eliminates spread of contaminants during transfer of containers before pupal harvest.

Because of the unsanitary conditions associated with insects raised on natural diet, rooms 6 and 7, where western spotted cucumber beetles are reared, are not part of the internal flow plan. Instead, the inside doors have been sealed, and the only access is from the outside. All needs for this species, except for environmental control and monitoring, are met independently of the artificial-diet program. For example, washing larval containers and preparing them for reuse is done under a terrace that runs the length of the building from the mechanical room to holding room 4. A sink in room 6 enables workers to harvest and sanitize eggs without risking contamination to other areas of the facility.

Room characteristics

Separation of species, and of different stages of the same species, is achieved in eight larval and adult holding rooms. Each room has individually controlled temperature, relative humidity, and lighting. Light fixtures are sealed to exclude insects. Each room has a hose bib and drain for washing walls and floors. Floor covering throughout the facility is seamless, troweled epoxy that extends 6 inches up the walls to provide coved corners for easy cleaning. Walls and ceilings are painted with an epoxy paint that resists abrasion and that is inert to sanitizing solutions. Portable racks for holding rearing containers facilitate inspection of insects, sanitation, and proper airflow.

Equipment

Equipment was selected to compliment facility design and flow plan and to provide a safe and healthy working environment. In the diet-preparation room, a 13-liter mixer is operated inside a fume hood to protect workers from allergenic dusts generated during preparation of diets. For larger batches of diet, an 80-liter, steam-jacketed

mixer is used. A hood in the pupal-harvest room removes spores from mold growing on diet and insect wing scales released during cleanup of oviposition cages. A collector in room 8 removes scales from oviposition cages of tobacco budworm moths.

Conditioned air is moved in the facility by two air-handling units located on the roof. One unit supplies high-humidity air (65%–80%) to adult oviposition rooms, while the other provides lower humidity requirements for larval holding rooms and work areas. All supply air is filtered through an absolute filter that removes 99.9% of all particles greater than 0.3 μm in size. Conditioned air entering the harvest room is exhausted to the outside and not returned to the rest of the facility because of the contamination potential. The air-conditioning system is pneumatically controlled from a panel in the hall. Environmental variables are monitored by a data logger/recorder programmed with high and low limits for each room. Significant variation from these limits is indicated by an audible signal, and detailed emergency procedures are outlined for workers who respond to the alarm. Operations of the air handlers, the chiller, the boiler, and the pneumatic control system are also monitored. If a breakdown occurs during nonworking hours, telephone equipment automatically dials an operator who identifies the problem and notifies the insectary manager.

Sanitation equipment provides a firstline defense against introduction of contaminants into insect colonies. Ultraviolet lamps, mounted on ceilings throughout the clean isolation area, are automatically turned on during nonworking hours to help reduce incidence of surface and airborne contamination. The autoclave has double doors for loading unsterile materials from the harvest-room side and for removal on the clean-storage side after sterilization is complete. The passthroughs were designed and constructed at Dow. Each is 0.8 by 0.6 by 0.8 m and is made of plywood covered with Fiberglas cloth and resin for strength. Doors on both ends are made of safety glass in an aluminum frame. The 15-watt germicidal-lamp fixtures installed inside the passthroughs are turned on for 30 minutes with a timer switch. To protect employees from skin or eye damage during illumination, spring-loaded switches shut off the lamp when either door is opened. The passthroughs allow containers to be moved between isolation areas and reduce the spread of contaminants. In fact, agar-plate counts have shown complete destruction of black-mold spores during operation of the passthrough.

Regulations and procedures

Regulations have been established to insure that sanitary conditions are maintained. No job is complete until all materials used are put away inside drawers or cabinets

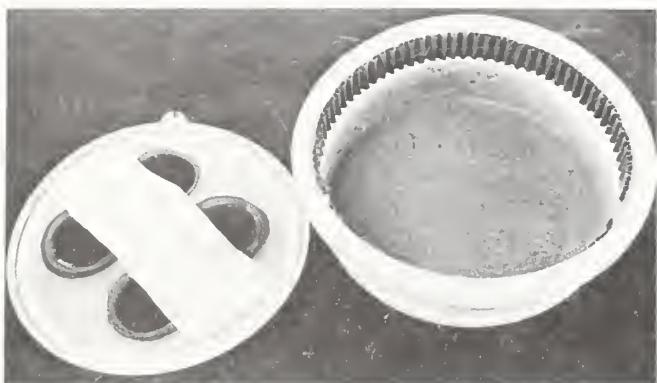


Figure 3.—Codling moth larval rearing container with diet, pupation sites, and surface-sterilized egg sheets taped to the inner surface of the lid.

and work surfaces are sprayed with disinfectant and wiped clean. Clean work areas are vacuumed daily and mopped with germicidal detergent twice a week. Access by employees to the clean area is restricted to only those who have duties there. Individuals exposed to disease organisms, for example when conducting field trials, are not allowed in the building. Lab coats and plastic aprons used during work in the harvest room are not allowed to be worn elsewhere in the insectary. Each isolation area has the materials and equipment required to complete jobs designed for those areas, so movement of items between areas is reduced. For example, there are three laboratory carts in the facility to service the various rooms in the major isolation areas. Materials or products are removed from carts and placed in passthroughs when being transported to another area. Larvae or adults that have escaped from cages are immediately discarded. If they are accidentally stepped on, the area is sprayed with disinfectant and thoroughly cleaned. Undesirable insects like cockroaches are controlled with sticky traps or boric acid, which do not affect colony insects.

Rearing procedures were developed to provide the most efficient use of space and time. For example, a better procedure for rearing the codling moth was developed. The problems with the old procedure were largely related to the square containers, loose lids, and paraffin-coating method. The new procedure is based on a new larval container. The container (fig. 3) has a modified snap-on lid that provides adequate gas and moisture exchange while preventing larval escape. Wax-paper strips containing eggs are taped to the underside of the lid, and newly hatched larvae drop to the artificial diet in the container and begin feeding. Prepupae leave the diet and pupate in a sterile, corrugated cardboard strip lining the inside

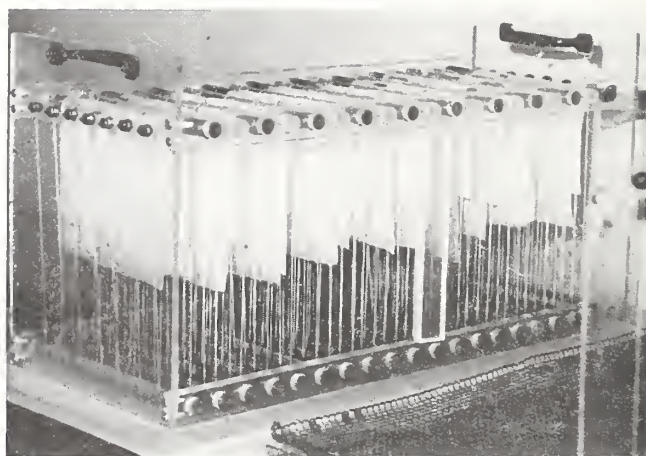


Figure 4.—Racks for surface sterilization of eggs laid on wax-paper substrates. Separation of egg sheets within racks insures direct contact with sterilant.

perimeter of the container. The strip is then rolled up and placed in a cage for eclosion and oviposition. The round container is easier to clean and sterilize than the old containers with square corners. Cleanup is also made easier by a new way to coat the diet with melted paraffin. While still hot, the diet is swirled 1-2 inches up the side of the container where it cools in a thin sheet. Melted wax is then applied to the diet surface by means of a soft camel's-hair brush, while the sheet of diet on the side keeps wax from touching the container. This new procedure has resulted in a fivefold increase in pupal yield and a 75% reduction in cleanup time. Fungal contamination of the diet has been totally eliminated.

Another procedure developed for the Dow program is a surface-sterilization technique for codling moth and beet armyworm eggs laid on wax paper. In the old procedure, layers of egg-laden wax paper were stacked and placed in a beaker containing a dilute bleach solution. But the waxy surfaces resisted wetting, making the disinfection process ineffective. The procedure also required frequent handling that damaged eggs or knocked them off the sheets. In the new procedure, egg sheets are placed in specially designed racks (fig. 4) that effectively separate sheets, insuring complete contact with the sterilizing solution. After sterilization, the racks are placed in two successive tanks containing clean water. Rinsing the sheets in this manner requires no running water; so about 11,000 liters/year are saved. After rinsing, the rack is removed, and the sheets are allowed to dry, a process hastened by separation of the sheets. This procedure also eliminates unnecessary handling of egg sheets.

Evaluation of the Dow Rearing Program

Once the new program began, the quality of all insects improved significantly, and yields became consistent and predictable. Disease symptoms were eliminated. Reduction in disease and dietary contamination allowed elimination of fungicides, Formalin (formaldehyde), and other antimicrobials from the diet. Symptoms of dietary deficiencies and inadequate environmental conditions that had previously been masked by disease symptoms became apparent, and steps were taken to eliminate their causes. Yields increased, in some cases dramatically, and have remained consistent from one generation to the next. So the number of containers needed to meet colony and research requirements was reduced for all five species at a significant savings in man-hours and raw materials. Because of more uniform development rates, testing

results became more reliable. Finally, health and safety hazards were significantly reduced by the use of equipment and procedures designed to provide a cleaner work environment.

The facility, equipment, and procedures in the new program allowed these improvements to be made. But the insectary manager made the program a success by coordinating activities, training personnel, anticipating and solving problems, and regularly improving the program. I discuss the role of an insectary manager in such a program in "The Insectary Manager."

Acknowledgments

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Industrial Insect Production for Insecticide Screening

By R. E. Wheeler¹

Introduction

The main objective of insect production in the pesticide industry is to rear enough organisms to use in screening for new insecticides. The insects may also be used to detect insect-growth regulators, sterilants, attractants, and repellents. In comparison to large industrial or government facilities that are mass producing insects to use in biological-control programs, pesticide-research laboratories usually have smaller rearing facilities, lower production quotas, and lower operating and equipment budgets. But the pesticide-research laboratories usually rear a broader spectrum of insect types. What types and number of species are reared depend on economic and environmental concerns and on trends in pest resistance to chemicals. And, whether a program is large or small, its success depends on having a facility designed for insect rearing and on having a management commitment to adequately staff and fund it. Quality insects can be produced only if these requirements are met. And a successful chemical screening program for insecticides depends on having quality insects. This paper outlines the guidelines and procedures we have developed at the Chevron Chemical Co., Richmond, Calif., for species selection; colony establishment, maintenance, and quality control; production and use; and product quality control. Following these suggestions should help insure a successful rearing program.

Selection of Species for Rearing

Industrial pesticide-screening laboratories test many compounds in several chemical classes of unknown pesticidal activity. Since insect species vary in their susceptibility to any one insecticide class, it is desirable to screen against many insect types to avoid missing a potentially useful insecticide. Selection of species for rearing must consider insecticide resistance and which target species and index species are feasible to use in the particular program.

Insecticide resistance

The selection of the degree of insecticide resistance in the insect population used to start a colony depends on the test methods used for the initial screen. When hundreds of unknown compounds are being screened for the first time, it is more practical to test compounds at a single high dose. If the insect population used to start a colony is too susceptible, too many false leads are obtained. For such single-dosage screening tests, it is desirable to select insect populations with insecticide resistance similar to that of the target-species populations encountered in agriculture. When initial or advanced screening methods use serial dilution dosages, the degree of insect resistance is less critical since dosage-response curves can be plotted, ranked, and compared to known standard insecticides.

Target species

When possible, the insects to be screened should include medically and agriculturally important target species. Selection can be based on the relative economic impact of the pests. But certain species are routinely used as test organisms because they produce a particular physiological, morphological, or behavioral response. For example, the yellow mealworm, *Tenebrio molitor* Linnaeus, and the reduviid, *Rhodnius prolixus* Linnaeus, produce highly quantitative and qualitative responses to juvenile hormone mimetics (Bowers and Thompson 1963, Wigglesworth 1969); and the American cockroach, *Periplaneta americana* Linnaeus, is useful for electrophysiological measurements of insecticidal activity (Narahashi and Yamasaki 1960a, 1960b, 1960c). Even parts of insects can be used—for example, antennae for electroantennogram studies in pheromone research (Schneider 1962, Davis 1973, Roelofs 1977).

Index species

In some instances, it is not feasible to rear the target species, because of time and expense or because its availability is limited by its distribution. So a substitute index species may be needed. The term "index species" refers to a species known to demonstrate susceptibility to standard known insecticides like that of a phylogenetically related target species. In California, because of quarantine restrictions, the western spotted cucumber beetle, *Diabrotica undecimpunctata undecimpunctata* Mannerheim, is commonly used as an index species for *Diabrotica* corn rootworms; these species have similar

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Table 1.—Species reared and the developmental stages used for screening potential insecticides at Chevron Chemical Co., Richmond, Calif.

Order and species	Stages used
Acari: Twospotted spider mite, <i>Tetranychus urticae</i> Koch	Adult.
Coleoptera:	
Confused flour beetle, <i>Tribolium confusum</i> Jacquelin du Val	Adult.
Egyptian alfalfa weevil, <i>Hypera brunneipennis</i> (Boheman)	Larval, adult.
Granary weevil, <i>Sitophilus granarius</i> (Linnaeus)	Adult.
Red flour beetle, <i>Tribolium castaneum</i> (Herbst)	Adult.
Sawtoothed grain beetle, <i>Oryzaephilus surinamensis</i> (Linnaeus)	Adult.
Western spotted cucumber beetle, <i>Diabrotica undecimpunctata undecimpunctata</i> Mannerheim.	Egg.
Yellow mealworm, <i>Tenebrio molitor</i> Linnaeus	Pupal.
Diptera:	
House fly, <i>Musca domestica</i> Linnaeus	Adult.
Yellowfever mosquito, <i>Aedes aegypti</i> (Linnaeus)	Larval, adult.
Hemiptera: Lygus bug, <i>Lygus hesperus</i> (Knight)	Adult.
Homoptera: Cotton aphid, <i>Aphis gossypii</i> Glover	Adult.
Lepidoptera:	
Beet armyworm, <i>Spodoptera exigua</i> (Hübner)	Larval.
Cabbage looper, <i>Trichoplusia ni</i> (Hübner)	Larval.
Tobacco budworm, <i>Heliothis virescens</i> (Fabricius)	Larval.
Orthoptera:	
American cockroach, <i>Periplaneta americana</i> (Linnaeus)	Nymph.
German cockroach, <i>Blattella germanica</i> (Linnaeus)	Adult.

biology and similar susceptibility to standard rootworm insecticides. The Egyptian alfalfa weevil, *Hypera brunneipennis* (Boheman), serves as an index species for the geographically restricted boll weevil, *Anthonomus grandis grandis* Boheman, and as a target species in itself.

In most cases, industrial insecticide-screening laboratories routinely rear at least seven insect species, including representatives of the orders Diptera, Coleoptera, Lepidoptera, Homoptera, Hemiptera, and Orthoptera; usually, one mite species is also reared (for examples, see table 1). Often, small colonies of other species, such as stored-product beetles and moths, are maintained and then enlarged when special projects call for their use.

Colony Establishment

Before an insect species is colonized, its life history, known rearing techniques, physical requirements, and probable insecticide resistance should be thoroughly studied. Important considerations are population source and population size.

Population source

Insects used to start colonies of agriculturally and medically important target species should be collected from their preferred host crop or natural habitat. And the

amount and kinds of insecticides used in the collection area should be ascertained. Often, other insectaries will provide a subculture with documented background information on insect origin, time in culture, and insecticide susceptibility (Dickerson et al. 1980). Use of a subculture should reduce the likelihood of introducing a pathogen or other contaminant into the culture. Field-collected material should always be reared in isolation to insure freedom from contaminants before introduction to the main insectary.

Population size

The sample size collected to establish a colony should be large enough to meet the minimum sustainable population size that will yield, after three generations, enough insects for preliminary standards testing. These preliminary data will indicate whether or not the selected population possesses the desired level of susceptibility to insecticides. The sample size is also guided by the fecundity of the species. As a general rule, 300 to 500 individuals at a given stage are enough to start a colony. To increase the chances of obtaining maximum homozygosity in this size of sample, only a single developmental stage should be collected. For highly vagile insects, this method minimizes chances of sampling two or more populations that have merged from diverse locations.

Rearing facilities

The insect-rearing system at the Chevron Chemical Co. uses a series of six rooms isolated from a central hallway by anterooms. Three of the rooms are used to rear several species that have similar environmental requirements. Even so, careful planning and procedures minimize the chance of disease transmission.

The "public-health insect" room (kept at 31.8° C) contains the German cockroach, *Blattella germanica* (Linnaeus); the American cockroach; the house fly, *Musca domestica* Linnaeus; and the yellowfever mosquito, *Aedes aegypti* (Linnaeus). Colonies are serviced by being transferred to an adjacent workroom through a small, sliding passthrough door. This workroom is maintained at a lower temperature than that in the "public-health insect" room to provide a more comfortable environment for the technicians.

The lepidopteran room contains cabbage loopers, *Trichoplusia ni* (Hübner); tobacco budworms, *Heliothis virescens* (Fabricius); and beet armyworms, *Spodoptera exigua* (Hübner). The larval stages of these species are reared in the same room. Pupae are removed to another isolated room for adult emergence and oviposition. This room is equipped with exhaust hoods for removing adult moth wing scales. The workroom for preparing artificial diet is also isolated from the rearing rooms. Both the diet-preparation and rearing rooms are designed for easy cleanup.

Other multiuse rooms contain several grain pest species; the Egyptian alfalfa weevil; and the lygus bug, *Lygus hesperus* (Knight).

Each of the other rooms contains only one species because of their space and environmental requirements and to prevent cross contamination. Greenhouses and outside beds provide space for raising host plants such as lima beans for the twospotted spider mite, *Tetranychus urticae* Koch; cucumbers for the cotton aphid, *Aphis gossypii* Glover; and alfalfa for the Egyptian alfalfa weevil.

Colony management

How a particular colony is managed depends on which stages will be used in testing. With rapidly reproducing species such as the cotton aphid and twospotted spider mite, schemes for colony and production management are the same. Insects in all stages of development are transferred to new host plants once a week after peak use demands have been satisfied. For those species whose adult stage is the one tested (table 1), a small part of the

adult yield is reserved to maintain the colony, and the rest is used to meet the production quota. Similarly, for those species whose immature stages are the ones tested (table 1), part of the harvest is reserved to maintain the colony.

Colony quality control

Colony homozygosity.—When the life cycle of the insect is longer than 1 week and a particular stage is needed once a week (as it is for house flies or lepidopterans), the colony must be divided into several developmental shifts. But dividing the colony creates a series of subcolonies. To maintain homozygosity among the subcolonies, we routinely reserve some eggs or pupae from one subcolony, place it in suitable cold storage for 1 week, and then backcross it with the next shift at a ratio of one stored to four new.

Biometric measurements.—Biometric measurements on egg production, length of development, size and weight of test stages, sex ratios, and yield of adults are important indicators of consistent rearing procedures. For the dosage-mortality responses to an insecticide to be considered accurate, these measurements must be within established ranges.

Environmental control

After many years of experience in designing environmental-control rooms for rearing insects, we have developed several design guidelines that are often overlooked in the basic design of commercial prefabricated environmental rooms. Equipment for establishing air quality (cleanliness, temperature, and relative humidity) should be housed outside the insectary. Preconditioned air should be continuously introduced into the insectary (not recirculated) so it creates a slight positive pressure at all room-entry points. This pressure reduces the possibility of airborne contaminants entering the insectary from outside through cracks, doors, or windows. The air-temperature-conditioning system (for both heating and cooling) should be one that gives the least amount of Btu's to achieve the desired temperature. To regulate the Btu inputs, solid-state proportional thermostats can be used to regulate steam-heating valves, electric-resistant load heaters, and refrigeration bypass valves.

Because environmental conditions in an insectary are not necessarily the same as the microclimate in the insect-rearing cage or container, sensing elements should be placed inside the insect-rearing cage to determine actual environmental conditions. And use of a programmable data logger to monitor important environmental conditions is

invaluable. Such systems provide computer-stored and printed data records and can automatically signal conditions that may endanger or disrupt the insect colonies.

Diet quality control

Quality control of artificial diet is maintained by closely following prescribed preparation methods and by using materials of known origin and consistent quality. If requested, most manufacturers will inform consumers of significant changes in composition or quality of their product. It is important to observe the stability of the shelf life for perishable components of diets, such as vitamin mixes and wheat germ. These items, particularly, should be placed in cold, dry storage to maintain maximum potency.

Natural diets consist mainly of host plants grown in greenhouses or outside beds. To obtain plants of consistent maturity, planting schedules should be shifted periodically to adjust for seasonal changes in growth rates. Perennial host plants such as alfalfa may require protection from insect and mite pests and from parasites and predators. The Chevron Chemical insecticide Dibrom² is very useful for cleaning up or eradicating pests from host plants. Dibrom is a broad-spectrum, contact insecticide exhibiting less than 2-day residual life on plants.

Contamination control

Facility design and material flow is especially important in keeping the insects as free from contamination as possible (see "Production of Insects for Industry. The Dow Chemical Rearing Program," by W. R. Fisher). Insect-rearing cages are also critical, and disposable ones should be used whenever possible. Disposable cages are particularly important for insects that are susceptible to pathogens and where rearing procedures are used that allow contamination of the insects, diet, and containers. Permanent or complexly designed cages should be constructed of stainless steel or polycarbonate plastics, which can withstand rigorous cleaning procedures such as autoclaving or steam cleaning.

Production and Use

Most industrial laboratories operate a complex initial screening schedule for herbicidal, fungicidal, insecticidal, and plant-growth regulator activity. At Chevron Chemical Co., 7 of the 18 insect species routinely reared are used in the initial screening that requires 2,000–4,000 individuals of a certain stage and age for each species on a

specified day of the week. Though the schedules and procedures for colony and production maintenance in a 5-day workweek often overlap considerably, the schedule for production maintenance is more flexible and can be changed according to how many insects are needed. The biology of the insect must be thoroughly understood if insect production and testing schedules are to be successfully synchronized. Those exogenous factors, such as temperature, humidity, and food, that regulate the rate of insect development and its quality must be identified and controlled.

The egg stage is a convenient point to synchronize production and utilization schedules. In our insectary, we use this stage to synchronize the production of yellow-fever mosquito larvae, Egyptian alfalfa weevils, cabbage loopers, and American cockroaches. The cotton aphid, western spotted cucumber beetle, and twospotted spider mite colonies provide a continuous supply of all stages of development. With colony maintenance on a 5-day workweek, pupae harvested from the house fly colony, which is maintained at 32° C, normally emerge on a Sunday morning. To provide 2-day-old adults for testing on Wednesday, some of the pupae are placed in adult emergence cages and incubated at 22° C. So adult emergence comes 1 day later than it does in the stock colony.

Product Quality Control

Quality control is embodied in every aspect of insect production from the physical design of the facility and production procedures to the insects themselves. For us, the most important quality characteristics are those showing that the insects have the required susceptibility to insecticides. The dosage-mortality response of the test insect to standard insecticides is the primary reference point for determining insect quality. Accordingly, one or two standard insecticides are routinely included in all tests on candidate insecticides. At regular intervals, a colony is monitored by tests of dosage-mortality response to a broad spectrum of insecticide types and classes. Throughout the entire period that a colony is maintained, a data bank containing standard data on insecticide dosage-mortality is kept and periodically analyzed for drifts in insecticide susceptibility. (Figures 1–3 illustrate how much colony susceptibility can fluctuate.)

The baseline LD_{50/90} values are first established after the data from the initial series of tests indicate that a new colony's standard insecticide response has stabilized. When a long-established colony's LD_{50/90} tends to shift beyond the standard deviation, close examination of the rearing procedure and biometric measurements may sometimes indicate reasons for the shift, particularly if there are changes in insect body mass and sex ratios. A trend toward increased susceptibility indicates genetic

²1,2-Dibromo-2,2-dichloroethyl dimethyl phosphate.

changes caused by lack of insecticide pressure on the colony or accidental introduction of susceptible insects into the colony. There are several ways to counteract such changes: apply insecticide pressure to the colony, infuse the colony with resistant field-collected specimens, or establish a new colony with the desired level of resistance. The method we use depends on the species and the seasonal availability of field specimens.

Occasionally, colony survival may be threatened by failure of environmental-control equipment, disease, invasion by other arthropods, or contamination. For example, in 1972 our cotton aphid colony was infested by a hymenopterous parasite. But we were able to reestablish this colony from a few (300) unparasitized aphids. Afterwards, dosage-mortality data from Dibrom tests with progeny from these individuals indicated an increase in tolerance. This increase continued for about 6 months; then the tolerance began to return to its previous level (fig. 1).

It is important that all colonies of a given species respond to chemical effects in the same way. For example, over the last 15 years, Chevron Chemical Co. has maintained a strain of twospotted spider mites resistant to parathion.³ When a change in the LD₅₀ response to parathion indicated increased susceptibility, the entire colony was subjected to parathion treatments until the dosage response stabilized (fig. 2). Likewise, how diet changes affect insect quality should always be examined. Several years ago, at Chevron Chemical, the agar component of the caterpillar diet was replaced by another gelling agent—Gelcarin HWG (Marine Colloids Division, FMC Corp., Springfield, N. J.). Examination of dosage-mortality data from caterpillars reared on the new diet indicated no changes in insecticide susceptibility (fig. 3).

Acknowledgments

I wish to express my appreciation to Jed Harrison of Chevron Chemical Co. for preparing the illustrations.

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Figures 1-3 follow on pages 245-247.

³O,O-Diethyl O-(p-nitrophenyl) phosphorothioate.

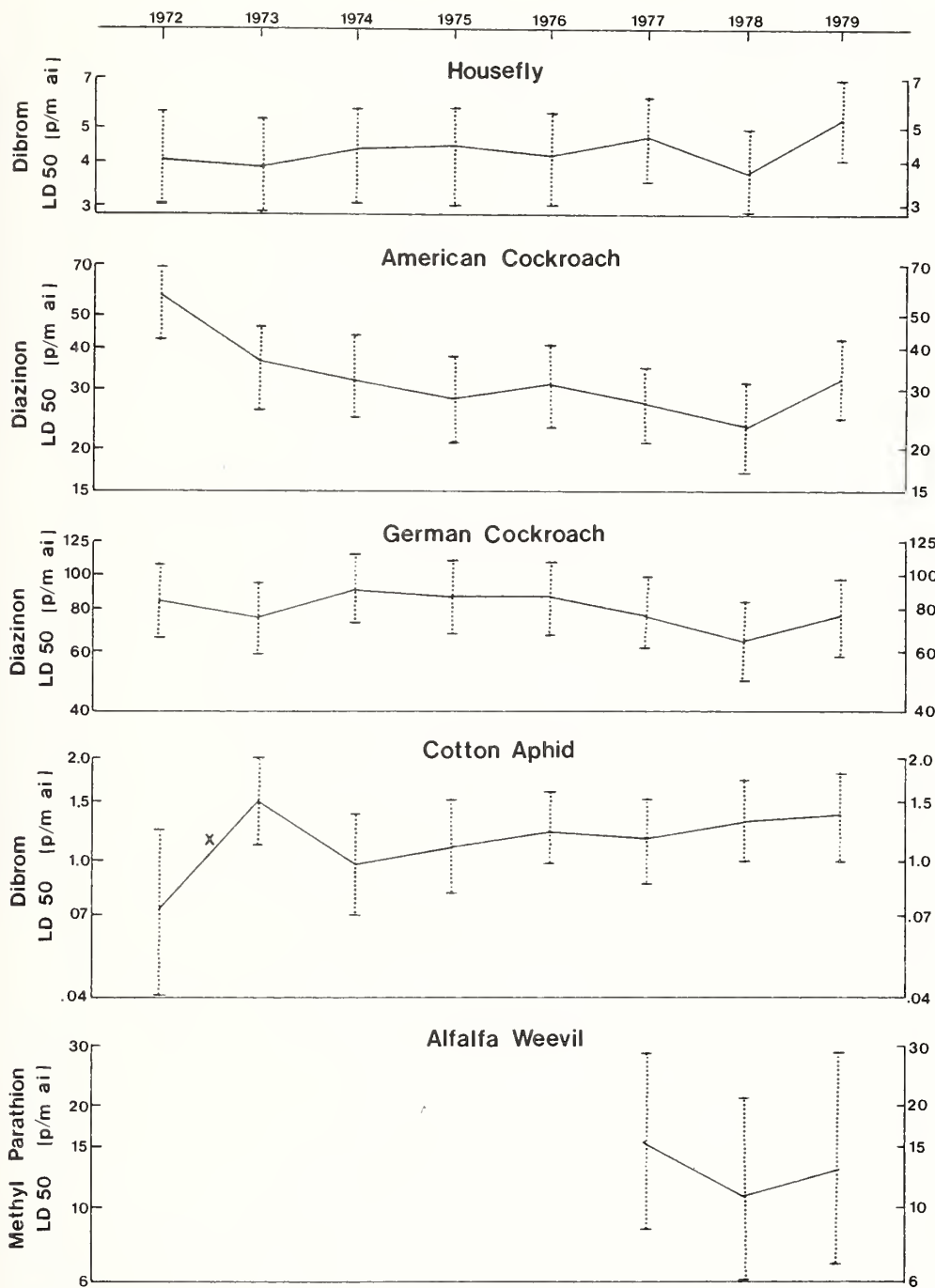


Figure 1.—24-hour LD₅₀ values for standard insecticides against various insects from 1972 through 1979. Applications: house flies—dosages (p/m ai in 70 μ l acetone) sprayed on each of 3 replicates of 20 adult insects (150 tests); American cockroaches—dosages (p/m ai in 70 μ l acetone) sprayed on each of 3 replicates of 10 fifth-stage insects (200 tests); German cockroaches and alfalfa weevils—dosages (p/m ai in 70 μ l acetone) sprayed on each of 3 replicates of 10 adult insects (200 tests); cotton aphids—aphid-infested cucumber leaves dipped in aqueous formulation (150 tests). Dotted lines fall between upper and lower 95% confidence limits. X=time when the aphid colony was parasitized by hymenopterous wasps and reestablished from 300 mother-aphid isolates.

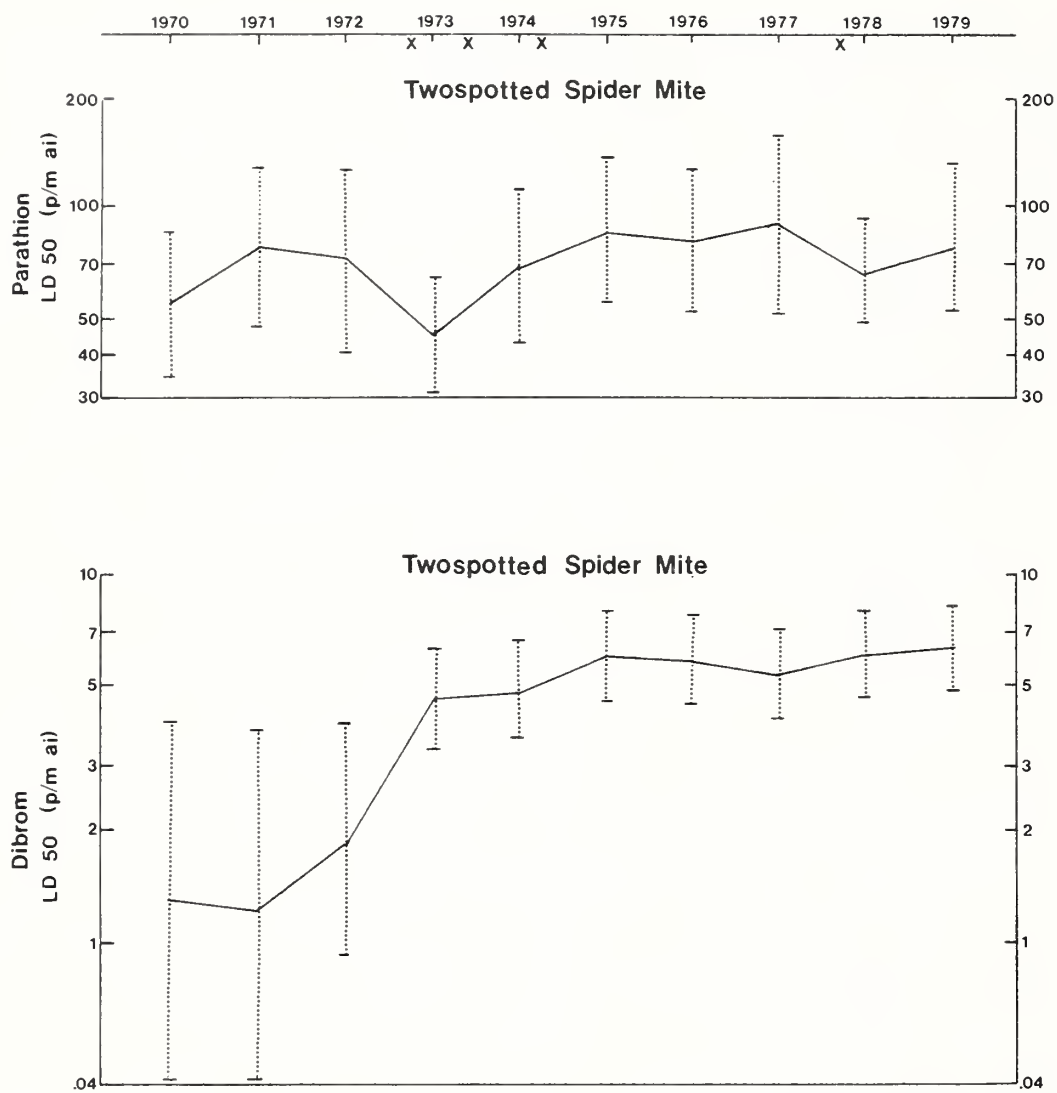


Figure 2.—24-hour LD₅₀ values for standard insecticides against adult twospotted spider mites from 1970 through 1979. Applications: mite-infested lima bean leaves dipped in aqueous formulations. Dotted lines fall between upper and lower 95% confidence limits. X (on horizontal axis)=time when entire mite colony was treated with a parathion spray (80 p/m).

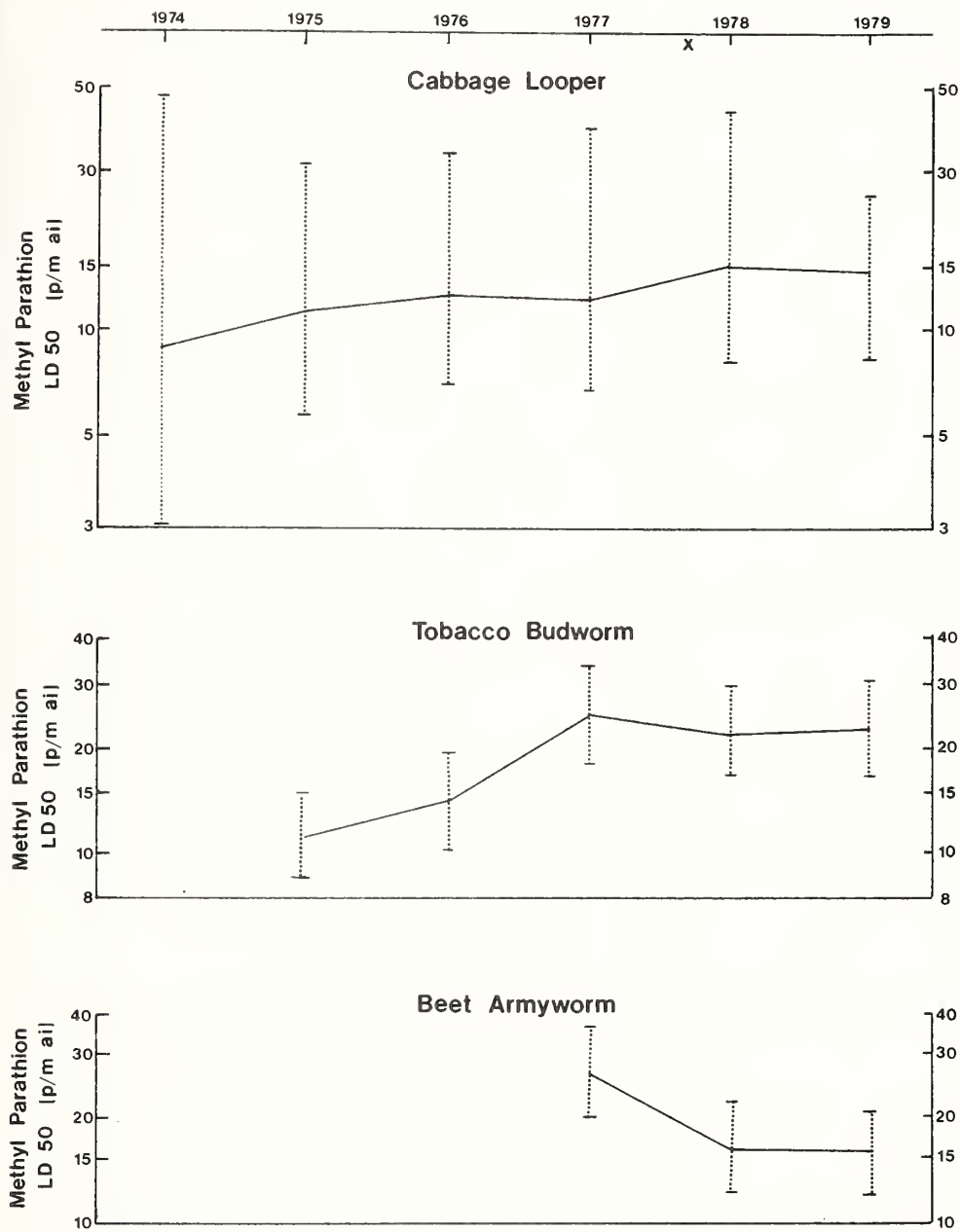


Figure 3.—24-hour LD₅₀ values for methyl parathion against third-stage larvae of various insects from 1974 through 1979. Applications: cabbage loopers—cucumber leaves were dipped in an aqueous formulation and then implanted with cabbage looper larvae (150 tests); tobacco budworms and beet armyworms—cotton leaves were dipped in an aqueous formulation and then implanted with tobacco budworm or beet armyworm larvae (150 tests). Dotted lines fall between upper and lower 95% confidence limits. X (on horizontal axis)=time when Gelcarin HWG was substituted for agar agar in the lepidopterous larval diet.

Mass Rearing the Cabbage Looper, *Trichoplusia ni*

By N. C. Leppla,¹ P. V. Vail,² and J. R. Rye³

Introduction

Laboratory-reared cabbage looper, *Trichoplusia ni* (Hübner), moths are needed to perform specific functions (as, for example, in the sterile-insect technique); and immatures are used as hosts for the production of parasites, parasitoids, predators, and pathogens (viruses, bacteria, fungi, protozoa, etc.). All stages are sources of biologically active compounds (hormones, pheromones, enzymes, etc.). The capability of producing thousands of adult cabbage loopers per day has been developed (Henneberry and Kishaba 1966 described this technology). Currently, however, more work is needed to devise better rearing methods that will produce moths that are qualitatively uniform and behaviorally effective. Developing better methods depends on a thorough analysis of past efforts, application of available technology, and expansion of research to determine the usefulness of alternative rearing methods. This paper discusses innovations now in use and some of the promising ideas currently being studied.

Rearing Techniques

Diets

Originally, diets for rearing cabbage looper larvae were adapted from some that had been developed for other species; these were based on agar and plant material (for example, the diets described by Bottger 1942, Adkisson et al. 1960, Berger 1963, and Shorey and Hale 1965). Which specific ingredients to use varies with what materials are locally available, the kind of mixing equipment, whether the diet ingredients can be used for other purposes (so that bulk orders would be appropriate), and personal preference of the scientists as limited by the rearing system. So products such as macerated host leaves, wheat germ, softened lima beans, ground pinto beans, and alfalfa meal have all been used in the basic

diet, alone or in various combinations. (See table 1 for lists of two of the least expensive diet regimes.)

The basic techniques of diet preparation have changed little. But procedures were simplified when the addition of chemical preservatives to the diet was developed to reduce the need for heat sterilization. Henneberry and Kishaba (1966) reviewed the antimicrobials suitable for use with cabbage loopers and presented detailed instructions for incorporating 1,800–2,000 p/m (parts per million) of sorbic acid and methyl *p*-hydroxybenzoate (methylparaben) preservatives into a diet of agar and alfalfa leaf meal. They also used Aureomycin (chlortetracycline) and Formalin (formaldehyde).

Table 1.—Two of the least expensive diet regimes for rearing cabbage looper larvae

Ingredients ¹	Diet	
	Pinto bean/ Wheat ²	Pinto bean/ wheat germ ³
Water ml . . .	3,560	3,400
Pinto beans	420	275
Wheat	128
Torula yeast	125
Wheat germ	200
Casein	100
Carrageenan	34	46
Ascorbic acid	15	13
Vitamin mixture ml . . .	12	12
Sorbic acid	3	4
Methyl <i>p</i> -hydroxybenzoate	5	8
Formalin	4	15
Tetracycline mg	250
Aureomycin (chlortetracycline) mg . . .	500

¹Unless otherwise noted, amounts are in grams (± 1 g).

²From R. Patana (Vail et al. 1972). Container—paper bag (16 by 19.5 by 32 cm deep). Temperature—26.7° C. Relative humidity—62%. Yield—average 130 pupae/container.

³Modified from W. W. Thomas (Vail et al. 1973) for use at the Insect Attractants, Behavior, and Basic Biology Research Laboratory. Container—paper cup (6.5 cm high by 11 cm diameter). Temperature—28° \pm 1° C. Relative humidity—65% \pm 5%. Yield—average 60 pupae/container.

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To rear six lepidopteran species, Patana (1969) used lima beans and carrageenan as principal dietary ingredients and transferred the medium into suitable containers with a pressurized diet dispenser. Burton (1969) used a pinto bean diet for the corn earworm, *Heliothis zea* (Boddie), and dispensed it into containers with an automated packaging machine. Either of these systems is suitable for rearing cabbage loopers.

Larvae

Since cabbage looper larvae are not especially cannibalistic, they may be reared in any vessel that will contain them and their diet, restrict microbial contamination, and maintain a suitable environment. Larval micro-environments are provided by balancing factors such as ambient climate, buffering capacity of the rearing containers, dietary constituents (particularly available moisture), and larval density and feeding behavior. Assuming that adequate environmental conditions are provided, selection of a suitable container depends on local availability, cost, amount of required handling, and space allocated for larval rearing. Vail et al. (1973) evaluated relatively inexpensive 16- by 19.5- by 32-cm-deep paraffin-coated paper bags by using different amounts of seven diets, varying the number of eggs, and incubating them in a constant environment. They found these to be successful containers, especially with the pinto bean/wheat diet shown in table 1. For paraffin-coated paper cups, the most popular larval rearing containers, Henneberry and Kishaba (1966) determined the best diet quantities, insect densities, and rearing conditions.

Pupae

Pupae are usually extracted from the rearing container by hand, and debris-laden silk is removed by washing them in a 1%-1.5% sodium hypochlorite solution (about 20% commercial bleach) for 10-15 minutes (Henneberry and Kishaba 1966). This procedure can be expedited if a portable washing machine is used for agitation (a sodium hypochlorite solution about 0.14%, is used for the 10-minute operation) and for automatic rinsing in water. After the pupae dry, a sorting machine may be used to position them for visual determination of sex (Wolf et al. 1972).

Oviposition and egg handling

Several kinds of oviposition cages have been tested, but the cylindrical type made of hardware cloth and wrapped with paper toweling remains the most useful (Ignoffo 1963). Henneberry and Kishaba (1966) tested one made of 0.64-cm-mesh hardware cloth that was 15 cm in diameter and 28 cm high. They found the best conditions to be 24°-29° C and 50% or higher relative humidity, with

48-60 moths per cage. Moths were fed a carbohydrate solution (1 g methyl *p*-hydroxybenzoate plus 50 g sucrose plus 5 cc unprocessed honey plus 0.1 g of ascorbic acid per 100 ml demineralized water) administered in cotton-filled cups. Precise ranges of tolerance have been established for the environmental factors that affect mating and oviposition in these cages (see, for example, Shorey 1963 and Henneberry and Kishaba 1967). Leppla and Turner (1975) demonstrated that light quality, particularly low intensity illumination at night, is important for maximum fecundity, and Petterson (1974) reported the use of fluorescent plant lights to increase egg production.

In large operations, the hardware-cloth cages release potentially hazardous insect scales into the insectaries. Carlyle et al. (1974) solved this problem by incorporating Plexiglas boxes with removable paper-toweling substrate into a scale-filtration system. A moth-collecting system (Ridgway and Whittam 1970) developed for the pink bollworm, *Pectinophora gossypiella* (Saunders), could also be adapted for use with the cabbage looper. But, in moderate-sized colonies, the oviposition cage made of hardware cloth is still the most convenient; scales may be controlled by placing the cages above a plenum connected to an industrial dust collector (Leppla et al., in press).

Selection of an oviposition substrate and surface-sterilization procedure for cabbage looper eggs depends on the overall treatment system and whether dry or wet eggs are placed on diet. Paper toweling is the most popular substrate; sometimes waxed paper and Teflon-coated or cornstarch-coated paper have been used because eggs can be removed more easily from these than from uncoated paper (Hoffman et al. 1968). In any operation, surface sterilization of the eggs with 10% Formalin (45-minute exposure, 45-minute rinse in water) or with 0.3% sodium hypochlorite (5-minute exposure, rinse with 10% sodium thiosulfate, and wash in water for several minutes) is recommended for elimination of viral and fungal contamination (Vail et al. 1968). The method using paper toweling and sodium hypochlorite has been incorporated into a liquid system using automatic egg collection and surface sterilization (Leppla et al. 1973).

Sanitation and internal security

Sanitation and internal security are basic to the maintenance of mass-rearing facilities for cabbage loopers and other Lepidoptera. A facility where large numbers of moths are reared should include at least three levels of quarantine (fig. 1). At the first level, adequate reception and decontamination areas must be available to insure that only pure materials are accepted into internal storage. At this level, females are screened for pathogens before their eggs are added to the colony. Only clean

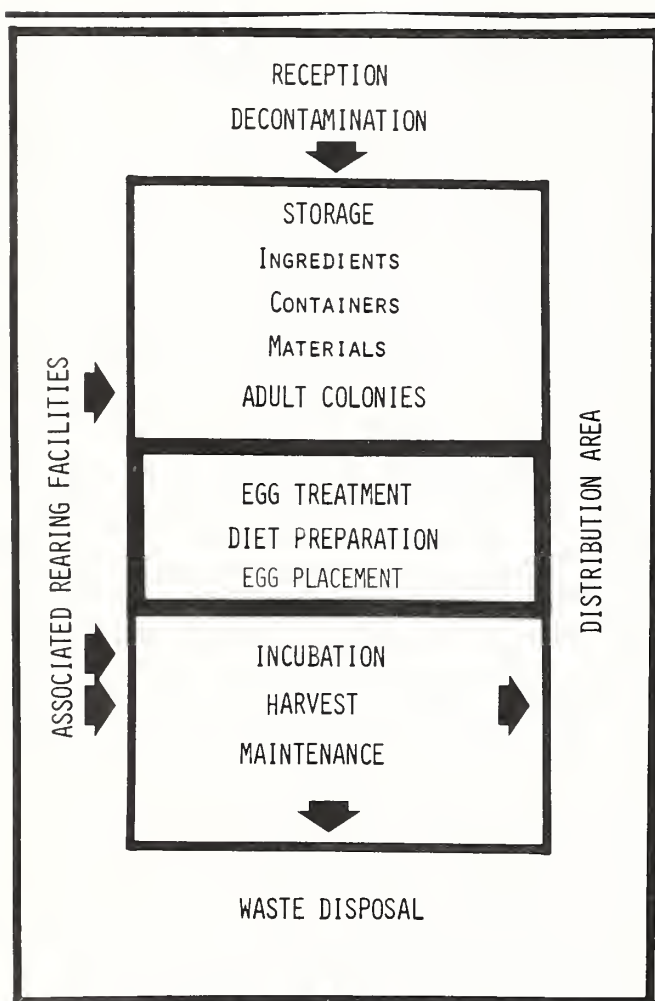


Figure 1.—Traffic pattern and levels of quarantine for a cabbage looper mass-rearing facility. Arrows indicate direction of exchange across thresholds and within the facility. The wider the boundary, the more internal security is enforced.

dietary ingredients, containers, and eggs are transferred to third-level areas where the diet is prepared and eggs are placed on it. Eggs and diet are sealed in containers before leaving this maximum security to begin incubation at the second level of quarantine. Pupal harvest and maintenance (purchasing, pupal sexing, packaging, minor equipment repair, etc.) also occur at the second level. Ancillary facilities may be used to house additional adults, incubate larvae, or provide general work and storage space at the first level of security (Leppla and Ashley 1978). Separate areas also at this level should be designated for product distribution and waste disposal.

Access is usually limited to rearing personnel to prevent contamination and maintain continuity in the work environment. Also, individuals should be physically isolated in specific work areas; or, if this is impossible, they may begin each day in places that require low levels of contamination and progress to less critical locations. For example, a person could prepare diet and place surface-sterilized eggs on it before harvesting pupae and handling adults. But routine disinfection is needed for effective isolation, and antimicrobials should be used when appropriate (Sikorowski 1975). Nutrient-agar plates may be exposed to the ambient air regularly to determine the effectiveness of this practice and to identify areas and procedures that become sources of contamination. If uncleanliness persists, the infested areas must eventually be fumigated with steam, formaldehyde, methyl bromide, etc., or disinfected and quarantined.

The degree of prophylaxis, routine disinfection, and decontamination depends on characteristics of the rearing facility. Absolute quarantine requires internal support systems (food, water, restrooms, communication) and traffic restrictions (locks, double doors, regulated air pressure, internal storage) that encumber efficient interaction among various parts of the facility. Too much exposed surface area and use of porous building materials precludes effective disinfection. Also, temporary facilities must be available when areas are vacated for decontamination. These measures reduce efficiency and increase building expenses; so they should be used only when absolutely necessary.

Current Research on Mass Rearing

Currently, researchers intent on improving mass rearing of the cabbage looper and other *Lepidoptera* are concerned mainly with quality control. The behavior and genetics of mass-reared insects must be appropriate to their particular function. To make certain that the product insects meet the requirements, techniques must be developed to routinely monitor vigor, competitiveness, irritability, durability, and overall responsiveness. An effective quality-control program depends on having standards of comparison for each variable. For the cabbage looper, most developmental standards have been established (for example, see Toba et al. 1970 for a rating system for larval diets and Smilowitz and Smith 1970 for a scheme to measure the growth of each larval instar). Setting behavioral standards is more complex because the appropriateness of behavior depends on the product insect's intended use (Boller 1972; Mackauer 1972; Chambers 1975, 1977; Boller and Chambers 1977; Webb et al. 1981). So only partial behavioral standards have been established (see Leppla et al. 1980 and Leppla and Guy 1980).

Other areas where research is improving techniques for

mass rearing moths include efficient preparation and dispensing of diet. For example, Harrell et al. (1973) and Sparks and Harrell (1976) have adapted machines developed for the food-processing industry. A diet that does not require cooking (for example, see Spencer et al. 1975, Leppla 1976, and Howell 1977) and a procedure for intermittent antimicrobial treatment (for example, see Chawla et al. 1967) could facilitate this automation. Progressive substitution and simplification of ingredients in the larval diet have been made possible by sophisticated nutritional studies (for example, see Chippendale and Beck 1968, Kishaba et al. 1968, Poitout and Bues 1972, Terriere and Grau 1972, Toba and Kishaba 1972, Dadd 1973, and Vanderzant 1974).

Researchers are also learning to control cabbage looper quality by controlling the rearing environment. For example, in an effort to increase rearing efficiency, Grau and Terriere (1967) studied how larval rearing temperature and dietary lipids influence the physical condition of resulting cabbage looper adults. They also quantified some of the complex interactions of environmental factors that affect larval development (Grau and Terriere 1971). Others (for example, Gothilf and Beck 1967 and Beck and Chippendale 1968) have studied how rearing environment affects larval behavior. The results of these studies have emphasized that the processes that make up the rearing environment are interrelated rather than independent.

Another area of current research is the automation of rearing procedures. For example, in a preliminary study to determine whether certain procedures in cabbage looper rearing could be automated, Stimmann et al. (1972) found that pupae could probably withstand mechanical harvesting. Other promising techniques have been developed for transferring moths without actually handling them (for example, see Seay et al. 1971 and Wolf and Stimmann 1971).

Development of new methods and materials to improve rearing efficiency has significantly reduced production costs. But implementation of these potential advancements depends on what can be done locally and practically. Automation and other advanced techniques are useful only if they complement functional rearing programs.

Conclusions

Large-scale cabbage looper rearing has been done at five locations (U.S. Department of Agriculture laboratories at Phoenix and Mesa, Ariz.; Columbia, Mo.; and Quincy and Gainesville, Fla.). Each of these locations has produced 25,000–100,000 pupae/week for at least 6 months. Efficiency of these facilities has varied with the quality of the facility and the availability of trained personnel. The

facilities with accurate, independent control of developmental environments, for example, have been able to prevent decomposition of the diet, promote uniform larval development, and therefore achieve greater pupal yields. Also, the facilities that filter airborne scales have been able to reduce the effort needed to care for adult colonies. So, in each of these pioneering rearing programs, 200–800 pupae have been produced per man-hour in 139–180 m² of space modified for all essential operations.

The need to provide a continuous supply of insects, as has been done in these facilities, has brought about an unusual synthesis between science and industry. Mass-rearing procedures are developed by teams of scientists (rearing specialists, other entomologists, and agricultural engineers) whose cooperation increases efficiency and decreases costs. They develop the procedures through understanding the growth processes of each insect stage, studying rearing ecology (climate, containers, competition, pathology, etc.), and investigating alternative materials and sources. Industry contributes the new materials and equipment specifically required for the new procedures.

The best way to organize these procedures, materials, and equipment into a rearing program is to conduct a systems analysis and implement its recommendations. A systems analysis is called for because mass rearing of insects is mainly an ecological discipline, one that must account for a host of interrelated variables. A systems analysis will consider all the primary variables that contribute to the efficient production of a quality product. And it will suggest the best ways to make use of the available procedures, materials, and equipment to reach that end. Of course, the system can only be as good as its available parts. Currently, then, since much work needs to be done on quality control in cabbage looper rearing, even the best programs can be depended on only for quantity.

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Section 6

Management of Insect-Rearing Systems

The purpose of this last section is to draw together and selectively expand the conference topics. In many respects, then, it recapitulates and unifies the overall program. So this section has two purposes: to consider important aspects of insect rearing that have not been elaborated earlier in the conference and to discuss concepts of management at levels ranging from small projects to massive international programs. Management concepts for insect-rearing programs have not, so far as I know, been discussed in such detail, so the authors were forced to think creatively and generate novel ideas. I encouraged them to do this not because they could produce perfect concepts, but because they are uniquely qualified to ask pertinent questions. Their answers, and your insightful modifications, should provide new testable alternatives for managing our science and technology.

Important aspects of insect rearing that have seldom been treated as separate topics are quality control, academic training, health and safety, and systems analysis. Derrell Chambers, well known for his previous contributions to quality-control theory, has collaborated with Tom Ashley to develop practical means for monitoring insect-rearing products and processes. Marian Brooks considers academic training from her perspective as a university professor; since curriculums specifically designed to prepare students for careers in insect rearing do not exist, her challenge has been to point out shortcomings in formal course work and to describe general skills that could be developed for handling problems in this field. Bob Wirtz volunteered to prepare a manuscript on human health and safety based on his recent national questionnaire on the subject; his findings, though based

on a pilot survey, indicate a need for concern about and documentation of the human pathogens and allergens encountered in insectaries. And Dave Akey, Bob Jones, and Tom Walton have invented the first comprehensive systems-analysis program for perpetual colonies. During the last 5 years, they have developed and implemented this information system by using it to manage colonies of biting gnats and mosquitoes.

Discussion of management principles and concepts begins with Chuck Schwalbe's and Tim Forrester's experiences in developing and operating the Gypsy Moth Project at Otis Air Force Base. Next, Bill Fisher probes the controversial question: What constitutes an insect-rearing specialist? He deals with the existing but previously undefined roles of centralized insectaries and their managers. My paper, based on a 10-year association with all levels of insect rearing, is specifically concerned with population-suppression programs. Our anchorman, Pat Patton, is one of today's most enterprising young managers. He currently oversees several massive insect-control programs and is eminently qualified to describe how important international politics can be to the successful conduct of insect-control programs.

Without the unifying influence of effective program management, current investments in insect production and utilization may be in jeopardy, if not wasted. We hope our efforts will encourage continued development of the expertise and technology necessary to effectively manage all sizes of insect-rearing programs and the control programs that they support.

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Putting the Control in Quality Control in Insect Rearing

By D. L. Chambers and T. R. Ashley¹

Concepts of Quality Control

Many studies have been done of concepts of quality control, including Feigenbaum (1961), Juran et al. (1974), Ott (1975), and American Society for Testing and Materials (1976). Quality control is essential to survival in today's competitive market, and insect rearing demands the same attention to quality control as any other industry. The concepts of quality control are also the same; these are:

1. "Quality Control" is a management procedure that develops, maintains, and improves quality.
2. The steps required in this process are setting standards, appraising conformance with those standards, acting on appraisal information, and planning how to improve the product.
3. The elements of quality control—"product control," "process control," and "production control"—are not the same, but they are closely related. They all develop information, have feedback loops, and control quality. But product control rejects faulty products, and production control maintains consistency of production output. Product control tells how well the product is conforming to specifications and standards of quality. It gives feedback so that a product's departure from established specifications can be corrected, or it eliminates substandard products. Process control tells how the manufacturing processes are performing. It controls these processes so that deviations from the product specifications will not occur as a result of variation in the processes. Production control regulates the consistency of production output, the numbers of items produced, and the timeliness of their production.

Industry's recognition of these concepts proceeded slowly; so did other quality-control functions such as product improvement and special process studies (acting on quality-control data to identify causes of defects). Progress was slowed by the reign of various enthusiasms, such as "statistical quality control," and of variations in

nomenclature, such as "quality assurance" and "system effectiveness." Struggles arose within corporations over the role, size, and management of quality-control divisions. But quality control has come a long way from an era characterized by product checking ("this one's good enough, this one isn't") to today's embracing of total quality control. For this concept, most experts on quality control have adopted tools and procedures to regulate the processes of production so that product quality will be insured through control of processes.

Entomologists often concentrate too much on production control while at best only controlling production processes and products. We still see quality control as an alarm and inspection system that oversees and intimidates production personnel. Instead, quality control should be used by production personnel to measure and control their own activities. This regulatory capability requires accurate information, which must be routinely gathered. This information must be used not only in decisions on whether to accept or reject the product but also in managing the processes that produce the products. In this paper, we will emphasize process control.

Process Control

Existing industrial quality-control techniques may be used for obtaining control over the processes used in insect production. These techniques will also help in the derivation of reasonable, attainable specifications for the products of those processes. Methods that estimate how well processes work include direct measurement of segments of a process (which requires setting aside a production unit for the term of its measurement), determining how well the product does what it is supposed to do (which is most meaningful but least quantifiable), and measuring how well the product conforms to its specifications (which lacks predictability, requires firm specifications, and does not provide process control). Two techniques, process-capability studies and process-control charts, would seem the best and most useful measures of our control over insect-production processes.

Process analysis

Process control requires that process analysis be applied to ongoing insect-production systems. Assume that the quality of performance by the insects being produced is acceptable; do you know why? Can you quantify how your production procedures result in acceptable quality?

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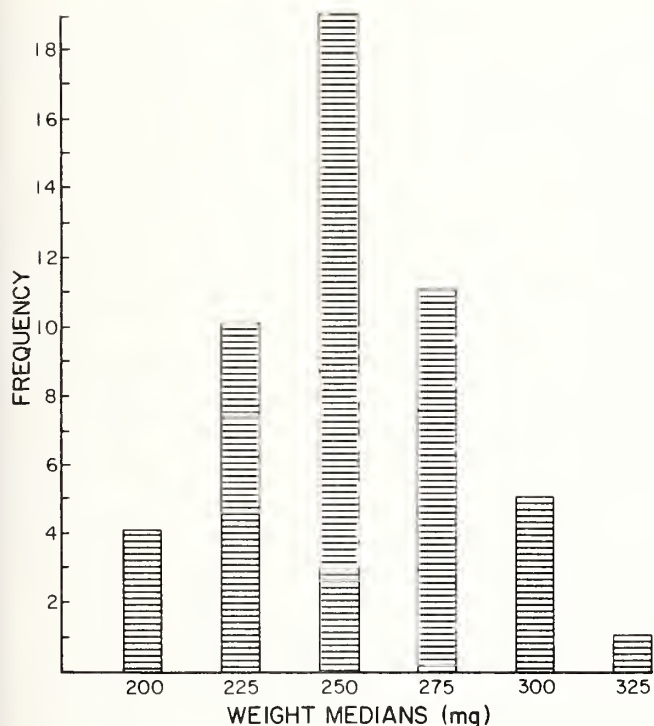


Figure 1.—Frequency distribution of the weights of 50 cabbage looper pupae. Such information can be used to identify normal variation.

Can you predict whether your processes will continue to produce quality insects? The first value of process analysis is its ability to identify causes of variability and to predict future performance. The second value is its ability to specify measurements of critical performance traits.

For example, a process might be a combination of people, equipment, supplies, and activities that results in a batch of pupae (one of a series of processes in an insect-rearing operation). The capability of that process is its ability to achieve measurable success in pupal variables (number, weight, diameter, color, etc.). The process capability of the associated operations is expressed in the measurable reproducibility of these variables. Variability is to be expected in, for example, the weights of pupae. But, if the production process is under control (not drifting or responding to uncontrolled influences), that variation will be random (not assignable to specific causes) and contained within limits. Quality-control engineering has found that a controlled process will result in measurements that fall within ± 3 standard deviations. This measure of dispersion is similar to familiar probability statistics.

Pupal weight is a variable of product quality. Measuring it tells us something about the acceptability of those pupae. Measuring it can also tell us something about the processes that produced those pupae. A continuing series of measurements will tell us about trends in the quality of the product and trends in the capability of the process to continue producing at that level of quality. Knowing what the quality is and has been is important, but knowing what it will be, knowing how much control we have over the production processes is important also. This is the process capability.

Process analysis begins with an examination of the variability of the process. A method used to examine the capability of a process is called the process-capability study. Its object is to define how much and in what way product quality varies. It accepts that a controlled process has a random variability (not assignable to specific causes) that should lie within ± 3 standard deviations. A process-capability study requires a large sample size—industrial practice suggests a sample size of 50. A frequency-distribution graph is made from this sample (fig. 1). If the sample is typical and the frequency distribution is normal, then process-control charting can safely be used. Figure 1 is a frequency analysis of the weights of 50 cabbage looper pupae and indicates the normality of these weights.

The process-capability study based on an analysis of frequency distribution describes how much and in what way the process varied at the time when the measurements were made. But it is more important to examine how much each production process varies sequentially. Frequency analysis is not well suited to such a sequential examination because multiple large samples would be too expensive. Also, frequency distributions are not easily interpreted, especially by technical staff or production crews.

Process-control charts

Process-control charts are an alternative to frequency analysis. They are chronological graphical comparisons of measured product characteristics. They can be used to detect and identify assignable causes of variation, and they can be used to warn of drift toward unacceptable quality due to nonrandom or assignable causes—they can predict developing problems.

Process-control charts represent the differences between means of small samples and the grand mean of a large sample and the differences between ranges of small samples and an average range (a range is the difference between the maximum and minimum values in a sample). A chart of means displays the central tendency of the average value of \bar{X} or \bar{R} from a sampling of n observa-

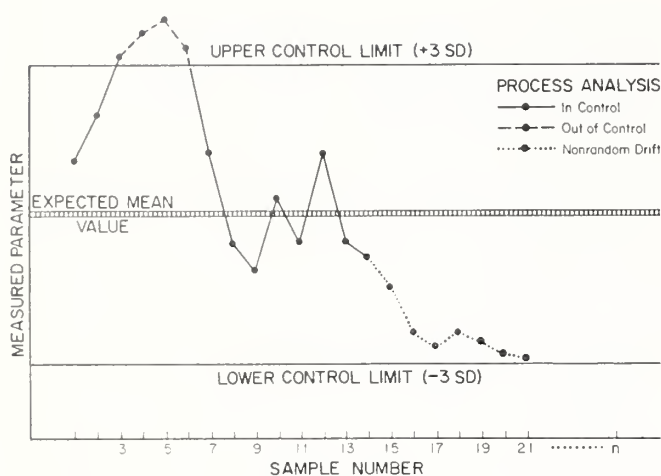


Figure 2.—Chart based on a hypothetical mean or range, illustrating acceptable and unacceptable trends in a measured variable.

tions of the measurements of a process; a chart of ranges displays the spread of these measurements. The two charts rely initially on the large sample drawn for the frequency analysis. The grand mean and the average range are placed on the control charts as the expected central tendency and spread. The limits of normal variation established above and below the central line are typically ± 3 standard deviations of the mean or range. The control charts are then used to plot sequential measurements of means and ranges made on small samples (usually 5 or 10) at regular intervals over a specific time. These measurements show trends in process controllability, and exceptional deviations can then be related to production activities or mistakes. When mean or range values exceed control limits, the process is probably out of control—a major problem exists on the production line. If variation is random and contained well within the control limits, control charting is needed to help maintain control. Even if the charted points fall within the control limits, non-random drift toward one of the limits (fig. 2) must be carefully interpreted.

For example, analysis of the frequency distribution of cabbage looper, *Trichoplusia ni* (Hübner), pupal weights (fig. 1) indicated random variation, so use of control charts was appropriate. We divided the sample of 50 pupae taken for the process-capability study into ten 5-pupae subsamples and determined a mean and range for each subsample. Then we calculated a grand mean and its standard deviation and an average range and its standard deviation. We used these values to plot the horizontal lines on the mean and range charts (fig. 3).

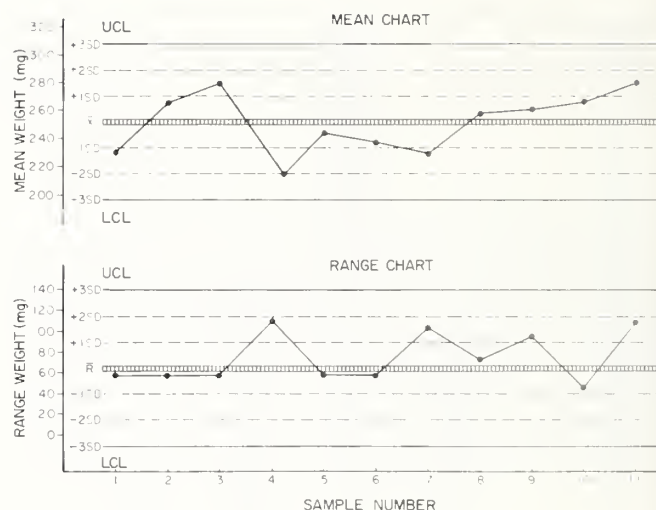


Figure 3.—Mean and range charts for weights of cabbage looper pupae for 30 days. Horizontal lines were determined from sample of 50 pupae divided into 10 subsamples. Data points were calculated from samples of 5 pupae collected on 11 successive dates. *SD*=standard deviation; range difference between the maximum and minimum values in a sample. *UCL*=upper control limit. *LCL*=lower control limit ($\pm 3SD$ in this example).

Next we took samples of pupae from the rearing operations at regular intervals. Although industry normally assumes sample sizes of 5 or 10 to be adequate, we measured 25 pupae and analyzed their weights in subdivisions of 5, 10, 15, 20, and 25 pupae to determine the best sample size. The analysis verified that a sample size of 5 or 10 is adequate. So we used only 5 of the 25 weights for each sample in plotting the points on the mean and range charts.

There were no alarming trends in either means or ranges, though there was one prominent deviation (sample No. 4); for mean pupal weight, the production process remained in control and contained well within ± 2 standard deviations. The deviation in means was seen by the production staff as they processed the pupae; deviations in range were not. Later, we analyzed variables influencing this process and attributed deviations in both mean and range values to larval crowding. This use of information from process analysis to solve production problems is an example of closing the feedback loop (see "The Closed-Loop System in Quality Control for Insect Rearing," by J. C. Webb). This use of the feedback loop was stimulating to the rearing staff and resulted in renewed analysis of the production process.

When it is not possible to calculate upper and lower control limits (± 3 standard deviations), use tables available

Table 1.—Comparison of control limits derived from data with those derived from a table¹

Value	Control limit (standard deviation)	
	+3	−3
Mean		
Data	314.4	192.6
Table	290.0	217.0
Range		
Data	136.7	(²)
Table	133.6	(²)

¹Calculations based on the pupal weights (mg) of 50 cabbage loopers in subsamples of 5 each; tables from Bicking and Gryna (1974).

²Three standard deviations below the average range value was a negative number.

(Bicking and Gryna 1974) for determining these values for both means and ranges. The control limits calculated from our data and those determined from a table were similar (table 1), so such tables will be fairly reliable.

Charts of means and ranges are normally displayed together, the mean chart above the range chart. In this way, production staff can easily follow the progress of their processes over time. This ease of preparation and analysis by technical staff is one of the greatest merits of process-control charting.

Conclusion

We advocate immediate adoption of control charting for each of your routine measurements within each of your processes. Examples in a fly-production plant might include fecundity, egg fertility, larval maturation rate, larval production rate, pupal size, percentage of eclosion, adult behavior, adult physiological variables, sterility assays, and release and recovery data.

Texts, handbooks, and manuals are available that describe many modifications of control charting. Our example is representative but simplified, and aspects such as sample size, frequency and variability must be examined. Based on an evaluation of how sample size affects a control chart's accuracy, we believe a sample size of 5 or 10 would be adequate for examining the process of pupal production, with weight as the measure, and days or batches as the frequency. But we set control limits at ± 3 standard deviations because of quality-control procedures established by industry. We feel that these limits may permit too much sample variation and suggest that ± 2

standard deviations might be more appropriate for pupal weights. The degree of variability permitted about an established average must be related to the expected performance of the reared insects.

We also believe that use of process-capability studies will aid in the difficult task of establishing useful standards, specifications, and tolerances. This task is especially challenging in programs of insect production and release because of the difficulty in adjusting unknown or partly known performance requirements to restricted flexibility in production processes. Standards and specifications are invariably compromises between these requirements and restrictions. Process analysis can provide data for making these compromises and feedback for regulating them.

Our experience with process analysis has encouraged us to adopt it broadly. We believe it offers information largely new to rearing facilities, information that will lead to new management capabilities and, therefore, to new levels in the professionalism of our efforts.

Acknowledgments

We thank N. C. Leppla, U.S. Agricultural Research Service, for providing the pupal weights used for the control charts.

Glossary

Assignable cause—an identifiable factor that contributes to variations in quality.

Central tendency—the expected or average value(s) of \bar{X} or R from sampling units of n observations.

Control chart—a chronological graphical comparison of measured product characteristics with limits reflecting the ability to produce, derived from past experience.

Control limits—limits on the spread from the central tendency (often ± 3 standard deviations), computed from the variations within sampling units, established to indicate the presence of an assignable cause of quality variation.

Control, types of

Quality control—integrative procedure that develops, maintains, and improves quality; it is the regulatory process through which quality of performance is measured and compared with standards and which takes action on the difference. It contains, but is not limited to, components of total quality control including product inspection, production management, and the tools, technologies, and assays of quality measurement.

Process control—regulation of the performance of production processes so that deviations from product tolerances and specifications do not occur.

Product control—regulation of the conformity of

the product to specifications and standards of quality.

Production control—regulation of the consistency, reliability, and timeliness of production output.

Feedback loop—returning output information to the beginning of a process for correcting discrepancies between intended and actual performance or for the maintenance of current process standards and procedures.

Process-capability study—analysis of process components (people, equipment, supplies, activities) to determine the inherent reproducibility of the process.

Quality—fitness for use.

Random variability—naturally occurring variation, not attributable to assignable causes in quality variation.

Specifications—measurable units and subunits of design.

Standards—design references.

Tolerances—acceptable limits of variation in specifications.

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Academic Training for Insect Rearing

By Marion A. Brooks¹

Introduction

The skills, training, and experience needed by people involved in insect rearing vary according to the kind of program—colonizing a new species, maintaining a small colony, or mass producing insects for specific uses—and the level of responsibility. This paper concentrates on what academic training will be useful to those who work, or intend to work, in insect rearing.

Training for Colonizing New Species

What must one know to colonize an insect species that has never before been successfully reared? The most basic requirement is, of course, to be an entomologist. But the courses an entomology student might take may not be pertinent. To be successful at colonizing new species, the entomologist should have courses that involve close observations of insects in their natural habitats. Knowledge of insect behavior in the field will help the entomologist know whether insects are behaving normally in the laboratory. The entomologist should be trained in the use of environmental controls so that he understands how to regulate light, temperature, and humidity. He should have a course in insect morphology so that he knows, for example, how to determine growth rates by measuring body parts. Likewise, he should have a course in statistical design and analysis that will teach him how to handle data such as growth rates. He should have a course in insect pathology so that he can recognize when the laboratory insects are diseased. He should have training in preliminary diagnostic procedures so that he can recognize when prophylactic measures are needed and what kind to use. Final identification of the disease organism may have to be done by a diagnostic laboratory after the colony has been rescued, so the entomologist needs training in how to collect and ship specimen samples. To be able to detect nutritional deficiencies, the entomologist should have training in insect behavior and nutrition.

In colonizing a new species, the entomologist must be versatile. He must be something of an engineer, a morphologist, a biometrician, a nutritionist, a pathologist, and a behaviorist. In actual practice, rearing a new

species may simply be a matter of consulting the literature on the rearing of similar species and proceeding by trial and error. But the student anticipating a career in insect rearing should follow a curriculum designed to support that career: animal nutrition and biochemistry; insect physiology, pathology, neurophysiology, behavior, ecology, and genetics; and comparative endocrinology. And the time to begin this course of study is not when starting a rearing program but early, even in the undergraduate years.

Training for Maintenance Programs

A second kind of insect rearing is the maintenance of stock cultures for support of small research or teaching programs. In such a case, a technician or student is trained by a professional entomologist to follow procedures and sequences already established by a pioneering investigator and improved by various modifications. Regular, routine care is specified, and the technical work demands strict adherence to a schedule. The need for a technician to have a broad background in entomology is lessened by the necessity of following instructions. But even here the operator needs to observe carefully to detect any deviations from normal and report them promptly to the supervisor.

Diseases caused by fungi and viruses are the greatest threats in small programs. On-the-job training should suffice to show the technician how to avoid contaminating the food with saprophytic molds or bacteria. The sources of viral infections are usually unknown, but again a few demonstrations of the appearance of insects diseased by viruses should be adequate to make this problem recognizable. The worker needs to be shown how to keep alert for the presence of mites, fungus gnats, small weevils, etc., all of which are potential troublemakers in an insect colony.

Although extensive formal academic training is unnecessary for maintenance work, an introductory undergraduate course in general microbiology would be helpful in teaching the beginner the principles of pure culture and the techniques of avoiding contamination. These comments pertain primarily to rearing Lepidoptera. Suitable modifications may be made for rearing aquatic insects or less fastidious orders such as Diptera or Blattaria.

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Training for Mass-Rearing Programs

Another kind of insect rearing is the mass-production program that is the subject of many of the papers presented in this conference. In these cases, the pioneering work has been done, the technicians have been trained, the engineering staff has been recruited, and the facilities have been built. Usually a single commodity is to be protected through the release of a parasitoid or of a pest species that has been altered in some way. The program's emphasis is on teamwork, engineering, and efficiency. Existing technologies are modified through statistical analyses, sound economic practices, and improved data collection. Improvements at these levels depend on the entomologists having advanced training and experience in insect physiology in its broadest sense.

For the mass-reared insects to be competitive when they are released into the field, their sensory physiology and behavior must not be radically changed. In some programs, it is desirable to maintain genetic variability; in others, quality control may depend on suppression of variability. Training in genetic measurements is a definite advantage for such work, but there is little evidence that such courses are available in entomology departments. While insect pathology courses are offered at many schools, students who did not make early plans to work in microbial control are rarely given any training in infectious diseases. It is obvious that academic course work should be broadened to include genetics and pathology in the training of any student who may work eventually in programs involving biological control through release of mass-reared insects and other organisms.

Academic Curriculums for Insect-Rearing and Biological-Control Programs

A tabulation of courses taught in entomology departments at 51 institutions (Pieters 1979) suggests that there is probably no formal offering of a course called insect rearing. Nor are there courses in nutrition, genetics, or endocrinology. These subjects are probably parts of courses entitled "physiology," "biology," or "behavior." Some of the listed courses useful in rearing insects are general entomology, biology, morphology, physiology, ecology, behavior, pathology, and biological control.

In anticipation that rearing programs will become more important in the future with increasing use of biological controls, entomology departments should encourage students to prepare for this kind of work. The feeding habits and nutritive requirements of many insects should be studied. Some students might have enough certainty about their future to be able to predict that emphasis on a given taxon—mosquitoes, for example—would be instructive. Some students might concentrate on a particular commodity, such as corn and its associated fauna. Specializations such as these should be supported by a broad program of insect physiology that would include many of the courses mentioned above.

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Health and Safety in Arthropod Rearing

By Robert A. Wirtz¹

Introduction

The colonization and rearing of insects and other arthropods are prerequisites for many studies in entomology and related fields. The mass rearing of arthropods is currently playing a critical role in the development and implementation of pest-control programs (Smith 1966). There are, however, potential health hazards associated with occupational exposure to arthropods. Extensive documentation exists on allergic responses to some arthropod products or bites (Frazier 1969, James and Harwood 1969, Ebeling 1975), especially to stinging Hymenoptera (American Academy of Allergy, Insect Allergy Committee 1965; Barnard 1973). Food inspectors, food handlers, and other workers have also developed allergies and dermatitis from exposure to material infested with arthropods. Little has been written, however, about allergies associated with arthropod rearing. Concern about this lack of documentation resulted in the formation in 1979 of an Insect Allergy Committee² sponsored by the Entomological Society of America. An initial goal of this committee was to identify and document the extent of health hazards associated with occupational exposure to arthropods.

Insect-Allergy Survey

In the fall of 1979, the Insect Allergy Committee mailed questionnaires to 135 educational, government, and private institutions in the United States. To survey institutions actively involved in arthropod rearing, the committee used the 1979 mailing list of the Insect Rearing Group's newsletter, Frass. Of those contacted, 82 responded; 48 (58.5%) of these reported at least one person with an allergy that coincided with occupational exposure to arthropods, their diets, or host animals; 32 (39.0%) reported that they had no work-related allergies; 2 (2.4%) reported having no occupational exposure. Allergies were reported by 113 people, 18 to 79 years old. The number of arthropod species encountered on the job

ranged from 1 to 76 with the mode being 2 (28.8%). People reporting allergies used some type of protective equipment routinely 25% of the time and as needed 60% of the time. The protective equipment used included gloves (35%), respirators/face masks (37%), head nets (15%), and others (clothing, exhaust hoods, etc., 13%). The complete results of the survey appeared in the Entomological Society of America Bulletin (Wirtz 1980).

The sources of reported allergies were divided into nine general categories (table 1). The Lepidoptera caused 69 (61.1%) of the reported allergies; most were reactions to moth or butterfly scales. The gypsy moth, *Lymantria dispar* (Linnaeus), was the most frequently implicated of the Lepidoptera, 32; then tobacco hornworm, *Manduca sexta* (Linnaeus), 8; pink bollworm, *Pectinophora gossypiella* (Saunders), 4; tobacco budworm, *Heliothis virescens* (Fabricius), 3; silkworm, *Bombyx mori* (Linnaeus), 3; greater wax moth, *Galleria mellonella* (Linnaeus), 3; unspecified moths, 4; and 1 or 2 each of 7 other species, 12. Of the 13 Coleoptera that caused allergies, 5 were weevils; 9 of the 11 Orthoptera causing allergies were cockroaches. Honey bees, *Apis mellifera* Linnaeus, were reported in 9 of the 12 cases for Hymenoptera. Mosquitoes caused five of nine Diptera allergies; most were reactions to adult scales. Eight of the Acarina allergies were attributed to mites and one to ticks. The dry ingredients in artificial rearing media caused most of the

Table 1.—Sources of allergies reported on questionnaires of the Insect Allergy Committee

Source	Response ¹	
	Number	Percent
Lepidoptera	69	61.1
Coleoptera	13	11.5
Hymenoptera	12	10.6
Orthoptera	11	9.7
Acarina	9	8.0
Diptera	9	8.0
Diets	6	5.3
Host animals	6	5.3
Other ²	13	11.5
Total	146	...

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²Committee members: W. A. Brindley; J. R. Gorham; A. M. Hammond; J. D. Hoffman; M. H. Weiden; and R. A. Wirtz, chairman.

¹Multiple responses from 113 people.

²One or more responses to: Aranea, Hemiptera, Isoptera, Neuroptera, silk, tissues, or hemolymph.

allergies related to diet. All allergic reactions to host animals were caused by rodents or rabbits. More than 68% of the people suffering allergies were exposed to the allergen daily; 12.4% were exposed weekly. Although eight people reported observing symptoms on first contact, and one person reported having had 20 years of exposure before the allergy developed, most estimates ranged from 1 week to 7 years with a mode of 12 months (20.8%). Most (77.0%) allergens came from airborne material (table 2). Sneezing and a runny nose were the commonest (68.1%) allergic reactions (table 3).

Of those persons reporting arthropod allergies, 28% stated that they had had to stop work or transfer; 49% had consulted a physician; and 47% said that the allergy had required some type of medication or medical treatment; 24% reported having used self-treatment (over-the-counter antihistamines); 25% were using prescription drugs; and 4% were receiving desensitization inoculations.

Table 2.—Suspected origins of allergic responses reported on questionnaires of the Insect Allergy Committee

Suspected origin	Response ¹	
	Number	Percent
Airborne material	87	77.0
Contact	72	63.7
Sting	8	7.1
Bite	4	3.5
No response	9	8.0
Total	180

¹Multiple responses from 113 people.

Table 3.—Type of allergic reaction reported on questionnaires of the Insect Allergy Committee

Symptoms	Response ¹	
	Number	Percent
Sneezing and runny nose	77	68.1
Skin irritation	70	61.9
Eye irritation	70	61.9
Breathing difficulty	38	33.6
Anaphylactic shock	3	2.7
Other ²	4	3.5
Total	262

¹Multiple responses from 113 people.

²Headache, nausea, faintness, fever.

Major Health Problems

Allergies and dermatitis

The insect-allergy survey showed that occupational health hazards associated with arthropod rearing usually affect the respiratory system, the skin, or the eyes. Rhinitis and bronchial asthma have resulted from exposure to silkworms (Inasawa et al. 1973, Tadani et al. 1974, Saakadze 1976, Fuchs 1979) and other moths (Stevenson and Mathews 1967; Smith 1973, pp. 405–407); and other occupational respiratory allergies have been caused by bees (Yunginger et al. 1978); flies (Ordman 1946, Ebeling 1975); cockroaches (Bernton and Brown 1964); beetles (Smith 1973, pp. 413–416; Ebeling 1975); locusts (Frankland 1953, Fuchs 1979); screwworms, *Cochliomyia hominivorax* (Coquerel) (Gibbons et al. 1965, Herrman 1966); mites (Wharton 1976, Fuchs 1979); mosquitoes, owlflies, termites, and the introduced pine sawfly, *Diprion similis* (Hartig) (Wirtz 1980). Airborne cuticular fragments, hairs, scales, and dried tissues and feces are abundant in most rearing areas. Some people can be extremely allergic to insect fragments (Perlman 1958, Marchand 1966, Fuchs 1979), and these allergens can sensitize through inhalation or contact (Bernton and Brown 1969, 1970). Two laboratories rearing Lepidoptera reported that 53% of their personnel developed allergies to moth scales even though exhaust hoods, protective masks, and laboratory coats were used routinely (Wirtz 1980). Sensitivity to the German cockroach, *Blattella germanica* (Linnaeus), has been correlated with the intensity and duration of exposure (Bernton and Brown 1967a). Insectary personnel, museum curators, food inspectors or handlers, and entomologists who have repeated close contact with arthropods are especially susceptible to allergies of arthropod origin. An absence of allergic symptoms does not mean that arthropods or their products are not causing sensitizations. Bernton and Brown (1967b) reported that when "nonallergic" people had skin tests with extracts from seven commonly reared insect pests of human food, 25% of the tests were positive.

Dermatitis, a well-documented allergic response to arthropods (Barnard 1966, James and Harwood 1969, Ebeling 1975), was reported by 63.7% of the people responding to the insect-allergy survey. Implicated sources include beetles (Scott 1962), moths and butterflies (Smith 1973, pp. 405–407; Kwangtung Occupational Diseases Prevention and Treatment Hospital and Chung Shan Medical College 1974; Perlman et al. 1976; Press et al. 1977; Guseinov 1977), cockroaches (Bernton and Brown 1964), locusts (Wirtz 1980), theraphosid spiders (Ratcliffe 1977), and mites (Alexander 1972, TerBush 1972, Krantz 1978). The twospotted spider mite, *Tetranychus urticae* (Koch), was the source of four of the nine allergies caused by Acarina. One respondent stated that everyone who

reared this mite in his laboratory had developed a contact allergy (Wirtz 1980). More than 20 dermestid species that damage insect collections have been recorded (Ebeling 1975), and a single larva can release over 3,000 urticating hastisetae (Okumura 1967, Mills and Partida 1976), which are capable of penetrating the skin (Smith 1973, pp. 413-416). Unlike the hollow stinging hairs of many Lepidoptera larvae, which may contain toxins, irritations from hastisetae appear to be solely mechanical. Hastisetae also cause eye irritation, either directly entering the eye or indirectly moving from the arthropod to the worker's hands and then to the eyes (Ebeling 1975, Mills and Partida 1976).

Envenomization

Since many arthropods can inject venom or salivary secretions by means of a stinger, mouthparts, or urticating hairs, they may be a serious health hazard to people rearing or collecting them (American Academy of Allergy, Insect Allergy Committee 1965; James and Harwood 1969; Barnard 1973). The stinging arthropods include Hymenoptera (bees, wasps, hornets, ants, velvet ants) and Scorpiones (scorpions). In the United States, *Solenopsis* spp. and *Pogonomyrex* spp. ants, and the *Centruroides* spp. scorpions pose the most serious problem to "nonallergic" people (Biery 1977, Frazier 1969). Allergic reactions to honey bee stings affect many beekeepers and their families (Yunginger et al. 1978, Lichtenstein et al. 1979). Honey bees and wasps are the most common causes of anaphylaxis (Barnard 1973, Gottlieb 1979).

Allergic reactions to any of the stinging arthropods can result in death. Every year, stings actually kill at least 50-100 persons in the United States (Lichtenstein 1977, U.S. Food and Drug Administration 1979, Gottlieb 1979). While some people become increasingly sensitive to stings, many have no forewarning that they might have anaphylactic reactions (Barnard 1973). Fortunately, only 11 of the 113 respondents to the insect-allergy survey listed allergies to stinging Hymenoptera: 9 to honey bees and 2 to the fire ants, *Solenopsis* spp. (Wirtz 1980).

The biting or piercing arthropods include the Thysanoptera (thrips), Anoplura (sucking lice), Heteroptera (true bugs and allies), Diptera (mosquitoes and biting flies), Siphonaptera (fleas), Acarina (mites and ticks), Aranea (spiders), and Chilopoda (centipedes). All of these, because they can inject salivary secretions or venoms through specialized feeding appendages, are a potential health hazard to insectary personnel (Frazier 1969, James and Harwood 1969, Ryckman 1979, Ryckman and Bentley 1979). As with stings, allergic responses cause most of the severe reactions to bites (Derbes 1971). Reactions vary greatly. Some people suffer no visible effects, not even swelling in the area of multiple bites, while others

react violently to a single bite (Scott 1966, James and Harwood 1969).

The larvae of more than 50 species of Lepidoptera can envenomize through urticating hairs. These specialized setae are usually hollow and connected to a gland that releases a toxin. The intensity of irritation varies with the species, sensitivity of the victim, and the exposed tissue (Smith 1973, pp. 405-407; Scott 1966). If urticating hairs get into the eye, they may cause blindness (James and Harwood 1969).

Chemical secretions

Arthropods are noted for the diversity of defensive chemicals they produce and their unique delivery systems (Roth and Eisner 1962, Eisner and Meinwald 1966). Defensive secretions are usually a mixture of these reactive, irritating, or toxic compounds: carboxylic acids, alcohols, aldehydes, ketones, esters, lactones, phenols, 1,4-quinones, hydrocarbons, steroids, and other miscellaneous compounds—for example hydrocyanic acid (Blum 1978). Many of these defensive exudates have constituents that significantly increase their effectiveness by facilitating spreading or penetration. Delivery systems range from localized exudation and reflexive bleeding to remarkably accurate defensive sprays (Eisner and Meinwald 1966). People rearing, collecting, or accidentally exposing themselves to these defensive chemicals may encounter risks ranging from temporary skin irritation to chronic exposure to suspected carcinogens. For example, certain tenebrionid beetles release a mixture of 1,4-benzoquinones when stressed. Even when few of these beetles are present, they can be detected by the quinone odor they give to an infested commodity. Their quinones are highly reactive compounds with suspected mutagenic and carcinogenic activity (Ladisch 1965, U.S. Environmental Protection Agency 1979). At least six other species of stored-products arthropods can also be detected by their odor, but the composition of these exudates is unknown (Wirtz, in press).

Parasites and pathogens

Many commonly reared or collected arthropods can parasitize humans (arthropodiasis) or be vectors of pathogenic organisms. Arthropodiasis may appear to be an unlikely health hazard under normal rearing conditions, but it can easily happen if sound laboratory practices are not strictly enforced. Over 100 species of fly larvae have been identified in cases of myiasis in man by James (1947), James and Harwood (1969), and Palmer (1970). Other cases have involved accidental enteric myiasis, canthariasis (beetles), scoleciasis (moths) and acariasis (mites) (James and Harwood 1969, Ebeling 1975). In one instance, a specimen collector got occupa-

tional "myiasis" by using an aspirator during insect collection; about 2 months after he had finished the summer collecting during which he used the aspirator 4-6 hours per day, 69 specimens from 4 insect orders were passed from the antrum of his sinus; apparently, the insects entered the sinus as eggs that had passed through the brass screen on the aspirator (Hurd 1954).

Arthropods are biological and mechanical vectors and intermediate hosts of human disease-producing organisms (for example, see James and Harwood 1969 and Smith 1973). Also, many human pathogens grow on synthetic rearing diets and other organic matter present in many insectaries. P. P. Sikorowski (see "Microbial Contamination in Insectaries. Occurrence, Prevention, and Control"), analyzing the problem of employee safety and microbial contamination in the insectary, concluded that humans are a major source of these pathogens. Filth flies and domestic cockroaches are often implicated as vectors of pathogenic organisms (James and Harwood 1969, Palmer 1970). Evidence for fly involvement is present for more than 60 human diseases (Greenberg 1973). At least 4 strains of poliomyelitis virus, 40 pathogenic bacteria, the eggs of 7 species of pathogenic helminths (Roth and Willis 1957, 1960; Frishman and Alcamo 1977), and several species of fungi and protozoa (Beatson 1976) have been isolated from wild cockroaches. Laboratory colonies established from wild populations should be checked carefully for human pathogens.

Generally, micro-organisms pathogenic to insects and used as active ingredients in microbial-control products will not harm man, other animals, or plants (Steinhaus 1957). Certain arthropod viruses, the baculoviruses, are surprisingly host specific and appear to be restricted to invertebrate hosts (Tinsley 1979). In 1973, the U.S. Environmental Protection Agency exempted arthropod virus from tolerance-residue requirements stating that it presented no known hazard to human health (Falcon 1976). But the desired viral agent must be carefully isolated and purified and then positively identified (Tinsley 1979). Bacteria pathogenic to insects are also being mass-produced after extensive field testing with no recognized hazards to human health or the environment (David 1975, Arata et al. 1978). Even though micro-organisms already tested seem to be harmless to humans and the environment, potential health hazards must be considered when new biological control agents are being developed and tested (Tinsley 1979).

Conclusions

Limiting contact with causative agents will effectively reduce health hazards associated with exposure to arthropods. Decreasing exposure to allergens, hazardous chemical and physical products, and pathogenic micro-

organisms is best accomplished by design or modification of laboratories, enforcing general sanitation requirements, and using protective equipment. If prophylaxis is impossible, people who continue to work with arthropods that cause them serious allergies should consider using a sting kit and getting desensitization inoculations (Lichtenstein et al. 1979). Because any strong allergic reaction is potentially serious, the sufferer should consult an allergist.

Identification and documentation of specific occupational hazards associated with exposure to arthropods should continue. This is the goal of the Insect Allergy Committee. An expanded survey has been distributed; the results will be used to inform insectary personnel, managers, and administrators what the hazards are. But the lack of specific allergenic fractions prevents the confirmation of work-related symptoms and the preparation of specific desensitization inoculations. Information from the expanded survey and from reliable research data will enable us to address the physical, ethical, and legal problems associated with human exposure to potentially hazardous arthropods and their products.

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Systems Analysis and Automated Data Processing in Insect Rearing

A System for the Biting Gnat *Culicoides variipennis* and Mosquitoes

By David H. Akey, Robert H. Jones, and Thomas E. Walton¹

Introduction

Quality-control tests are being used increasingly to determine the fitness of insects produced for laboratory research or in mass-rearing programs (Chambers 1977). Daily records must be kept of environmental variables, their parameters, and changes in nutritional regimens; other pertinent data concerning rearing and quality control must be recorded for each generation or production run. The recordkeeping and analyses needed to process the data are an added burden to the task of rearing insects. To alleviate this problem, we enlisted the help of personnel in the U.S. Department of Agriculture, Science and Education Administration, Administrative Management, Communications Data Services Division (CDS) and explored how systems analysis can be used for gathering, analyzing, and retrieving data associated with the production of insects.

Systems analysis has been defined as "the approach to a system that is the opposite of trial and error. All influences and constraints are identified and evaluated in terms of their impact on the various parts of the system" (FitzGerald and FitzGerald 1973). Put another way, systems analysis is "the examination of an activity, procedure, method, technique, or a business to determine what must be accomplished and the best method of accomplishing the necessary operations" (Sippl and Sippl 1972). The increasing sophistication of computers in the last 20 years has contributed to the increasing sophistication of systems analysis. In fact, it is frequently viewed as part of a broader concept of a management information system that provides information used by management for decisionmaking (Lucas 1974, Youssef 1975). Personnel responsible for rearing insects should view systems analysis as a way to organize insect-rearing operations in efficient and logical sequences. Such organization facilitates timely analysis, makes data retrieval possible, and aids decisionmaking. Computers are tools used to achieve these goals and should be

viewed as "devices that can be harnessed to serve information needs and improve organization efficiency" (Alexander 1974). For more details about systems analysis and related technology, see Bingham and Davies (1972) or Gore and Stubbe (1975).

Insect rearing can be viewed as a business that can benefit directly from the use of systems analysis and of automated data processing (ADP) with computers. As an example of how such an operation works, we present the methods used to apply systems analysis to the production of colonies of the biting gnat *Culicoides variipennis* (Coquillett)—the primary vector of bluetongue virus in the United States—and of several colonies of mosquitoes. Although the procedures detailed here were designed for these Diptera, they can probably be used readily with most insect colonies. To our knowledge, this is the first time that systems analysis and ADP have been applied to insect rearing.

Methods and Materials

We began the application of systems analysis to insect rearing by having the rearing operation reviewed by personnel from the CDS to determine if the proposed project was feasible. A systems analyst and a computer programmer then worked with us to determine what portion of the rearing operation would be easiest to incorporate into an information system and to select components of the rearing operation for inclusion in the system. We decided to limit the first information system (the one described in this paper) to data that could be recorded on a single 80-column coding form. To simplify this presentation, all examples refer only to *C. variipennis* unless otherwise stated.

C. variipennis gnats were reared with the methods of Jones et al. (1969), and their size was monitored by dry weight and wing length (Akey et al. 1978). Techniques for rearing mosquitoes generally followed those described by Gerberg (1970). The larvae were reared in 38- by 23- by 6-cm white enamel photographic developing trays and fed powdered rabbit food and/or a high-protein chow (Purina Special Steer 32, Ralston Purina Co., St. Louis, Mo.) or feed supplement (Jones et al. 1969). Adults were held in 30.5-cm³ cages.

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All data were recorded manually and then keypunched on 80-column cards with IBM (International Business Machines Corp., White Plains, N.Y.) machines. An IBM 370/168 computer, associated peripherals, and operating systems at the U.S. Department of Agriculture, Washington Computer Center, Office of Automated Data Systems, were used for processing and storing data. A Teletype (Teletype Corp., Skokie, Ill.) model 43 teleprinter with a Com Data (Com Data Corp. Skokie, Ill.) model 302F2-33 modem (a device that changes computer-compatible digital signals to telephone-compatible analog signals and vice versa) was used as a terminal for time-share operation (TSO). It was operated at half-duplex (unidirectional communication over a line) over voice-grade telephone line at 300 bauds. The computer was operated with both standard software (collections of computer programs) and with programs written specifically for this project. Before data on quality control were keypunched, preliminary analyses were performed with an HP9830 (Hewlett-Packard Calculator Products Division, Loveland, Colo.) calculator system programed with either the 98930A Stat Pac 1 or the General Statistics Pac. All other analyses were performed with the IBM 370/168 programed with the Statistical Analysis System (SAS, SAS Institute, Raleigh, N.C.; At this time, SAS can be run only on IBM mainframe computers).

Entry of Data

Flow charts and source and record documents

The first step in devising a system for data entry was to review the daily rearing operations that had been listed and ordered in logical sequences on four check sheets;² one of these is shown in fig. 1. In the second step, we reviewed the data-collection forms used with the check sheets (for example, fig. 2). Next, data recorded merely as a convenience were separated from essential data. Also, data that were not permanently recorded were reevaluated to determine if they were needed. All essential data were divided into groups of related fields (particular categories of data treated as a set or group). The fields were arranged in logical sequences for data collection and recording.

In the third step, we used a specially designed record document to list the fields. Headings were typed on a general purpose 80-column coding sheet, 8½ by 14 inches, that was revised several times from its conception in 1974 until a final document was approved in 1977. After 2 years of use, a more convenient form was printed on

8½- by 11-inch paper (fig. 3). The fields on this document are described in the procedure on coding data (appendix A). Quality-control data on insect size were recorded on two other record documents, one for pupal production and one for wing length and dry weight.

Data coding and editing document

The fourth step was the development of a data-coding system that was simple but comprehensive enough to cover the variations that occurred in our rearing operations. Additionally, we needed an editing document that gave the ranges, limits, and code types (alphabetic, numeric, or alphanumeric) for each field that was coded (appendix A). The combined data-coding and editing document was used as a guide for proper coding and recording of data about insect rearing. It defined the permissible relationships between codes for different fields and was operated as a program in the computer so that data entered were tested against the editing criteria. Data that did not meet the criteria were listed in the printout of the editing program and corrected if wrong; data that did not meet the editing criteria but were correct were left unchanged. Examples of the codes and permissible values were posted for use by the technicians who took care of our insect colonies. Some coded data are shown in fig. 3. Data collected on the record document (fig. 3) were keypunched on 80-column cards, entered in the computer, and stored in data files (collections of related records) on magnetic tape. Quality-control data were reduced to mean, standard error, etc., with a calculator before entry into the computer. The computer program prepared by the systems analyst to perform the editing listed in appendix A is shown in appendix B.

Retrieval of Data

Retrieval-request document

To produce reports on insect rearing, we first prepared a retrieval-request document (appendix C) for the systems analyst. This document was used as a guide for preparation of the required software. It listed the items of data input necessary for the software to generate the requested report. Also, the document listed examples of hypothetical output.

Retrieval request reports via TSO terminal

The retrieval programs were written to make use of SAS, a versatile and easy-to-use software computer package. Retrieval requests were made from a terminal. The desired SAS retrieval program was operated in an edit mode that allowed input or changes to be made in the program from the terminal; fig. 4 is an example of a

(Continued on page 279.)

²In computer terminology, these are known as flow charts in the form of check lists.

DAILY MASTER CHECK SHEET

*C. variipennis*Colony 000-AASHEET NO. 38DATE 75328FORM 005

DATE	328	329	330	331	332	333	334	335	336	337	338	339	340	341
INITIALS for daily work	EP	JH	JH	EP	EP	EP	EP	JH	JH	JH	JH	EP	EP	EP
Time Start	0430	0400	0400	0800	0400	0800	0800	0400	1000	0900	0400	0400	0800	0730
Record pan temperatures	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Light timers- lights on	X	X	X	X	X	X	X	X	X	X	X	X	X	X
timers in use- setting correct	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pupae picked- every other day	X	-	X	-	X	-	X	X	-	X	-	X	-	X
Larval Pans Setup* Mon,Wed,Fri	3	-	3	-	3	-	-	3	-	3	-	3	-	-
Holding Pan Setup Mon,Wed,Sat	/	-	/	-	-	/	-	/	-	/	-	-	/	-
Larval Pans Dumped Mon,Wed,Fri	3	-	3	-	3	-	-	3	-	3	-	3	-	-
Check Pan Water level - Daily	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Correct filming Daily	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Determine food requirements	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Add food to larval pans; bacterial starter	X	-	X	-	X	X	-	X	-	X	-	X	X	-
NB	X	X	X	X	X	X	X	X	X	X	X	X	X	X
dry; Alf, K, J*	K	J	K	J	K	K	J	K	J	K	J	K	K	J
Larval pan record sheet	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adult setup record sheet	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adult holding record sheet	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Time out	1400	1030	1600	0900	1330	1000	1100	1300	1300	1400	1000	1400	1000	0400
Water circulators on A and B	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Check reservoirs pump on	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pump running	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Water level	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Eggs Placed - Mon,Wed, Fri	X	-	X	-	X	-	-	X	-	X	-	X	-	-
Refrig. Temp.	6.0	6.0	5.5	6.0	6.3	6.3	6.3	6.2	6.5	6.5	6.0	6.5	6.5	6.0

*Letter or number

Figure 1.—Original check sheet to record insect rearing operations used for the primary *Culicoides variipennis* colony. NB is nutrient broth food; Alf, K, J are dry powdered foods—see Jones et al. (1969).

ADULT HOLDING RECORD
C. variipennis
 Colony 000-AA

SHEET NO. 16
 DATE 7-4-88
 FORM 003

DATE	288	289	290	291	292	293	294	295	296
Egg estimates* Tu(C);F(A);Sun(B)	C 40,000	-	-	A 40,000	-	B 30,000	-	C 50,000	C 500
Dead flies removed -Daily-	X	X	X	X	X	X	X	X	X
New cage set up* M(A);W(B);F(C)	-	B	-	C	-	-	A	-	B
By use of flies from*	-	1089- 1093	-	1094- 1096	-	-	1097- 1101	-	1102- 1108
Blood fed M(A);W(B);F(C) Rabbit no. <u>1</u> *	-	B	-	C	-	-	A	-	B
Change: 2 sugars and 1 water-Daily*	X	X	X	X	X	X	X	X	X
Place egg dish* M(C);Th(A);Sat(B)	C	-	A	-	B	-	C	C	-
Notes**									
DATE	297	298	299	300	301	302	303	304	305
Egg estimates* Tu(C);F(A);Sun(B)	-	A 40,000	-	B 60,000	B 1,000	C 50,000	C 10,000	-	A 30,000
Dead flies removed -Daily-	X	X	X	X	X	X	X	X	X
New cage set up* M(A);W(B);F(C)	-	C	-	-	A	-	B	-	C
By use of flies from*	-	1109- 1116	-	-	1117- 1120	-	1121- 1125	-	1126- 1133
Blood fed M(A);W(B);F(C) Rabbit no. <u>1</u> *	-	C	-	-	A	-	B	-	C
Change: 2 sugars and 1 water-Daily*	X	X	X	X	X	X	X	X	X
Place egg dish* M(C);Th(A);Sat(B)	A	-	B	B	C	C	-	A	-
Notes**									

Blood Feeding Host _____
 Temperature & Relative Humidity _____ °C / _____ %RH
 Photoperiod: Light _____ (hr)

*Use letters A, B, or C, or "All"
 **Indicate under "Notes" Maintenance of old cage.

Figure 2.—Original data-collection form for recording holding and maintenance data for adult flies for the primary *Culicoides variipennis* colony. BF is blood feeding of flies from cages (4-digit numbers in left column) that were combined into cages A, B, or C used to propagate the colony.

Form WR-710 (12/79)

33-00311

Figure 4.—SAS pupal production program. Arrows (not part of program) indicate data inputs that were allowed to stay as presented or changed to conform with needs of user.

```

1;
COMMENT PROGRAM #1;
* CHOOSE THE TYPE OF TIME UNIT ON RECORD;
* FOLLOWING THE MACRO MAC1 STATEMENT;
* CHOOSE ONE: WK MON QTR YR;
MACRO MAC1
→ WK
%
* CHOOSE TYPE OF CUMULATION FOLLOWING THE MACRO MAC1A STATEMENT;
* CHOOSE ONE: CUM_WK CUM_MON CUM_QTR CUM_YR;
MACRO MAC1A
→ CUM_WK
%
* CHOOSE TIME UNIT OF REPORT FOLLOWING THE MACRO MAC1B STATEMENT;
* CHOOSE ONE: WEEK MONTH QUARTER YEAR;
MACRO MAC1B WEEKZ
* CHOOSE COUNTING METHOD(S);
* FORMAT MAY BE MULTIPLE (METH= D', METH= D OR METH= E ETC.);
* PLACE FOLLOWING MACRO MAC1C STATEMENT;
MACRO MAC1C
→ METH= D' OR METH= E OR METH= F
%
* SELECT START OF JULIAN DATE RANGE;
* PLACE AFTER MACRO MAC1D STATEMENT;
MACRO MAC1D
→ 76205
%
* SELECT END OF JULIAN DATE RANGE;
* PLACE AFTER MACRO MAC1E STATEMENT;
MACRO MAC1E
→ 76247
%
* SPECIFY COLONY OR COLONIES DESIRED;
* ENCLOSE SC VALUE IN SINGLE QUOTES;
* PLACE AFTER MACRO MAC1F STATEMENT;
MACRO MAC1F
→ SC='AA'
%
* SPECIFY IF PLOTS ARE DESIRED FOLLOWING THE MACRO MAC1G STATEMENT;
* 1=PLOT 2=NO PLOT;
MACRO MAC1G
→ 1
%
* THIS MACRO CONTAINS THE PLOTS;
MACRO MAC1H
PROC PLOT DATA=READY; FORMAT SC $SCC. METH $METH.; BY SC METH;
PLOT TOTAL*MAC1A= * ; TITLE PUPAE COUNTS BY MAC1B;
PROC PLOT DATA=READY; FORMAT SC $SCC. METH $METH.; BY SC METH;
PLOT CUM_TOT*MAC1A= * ; TITLE CUMULATIVE PUPAE COUNTS BY MAC1B;
PROC PLOT DATA=READY; FORMAT SC $SCC. METH $METH.; BY SC METH;
PLOT TOTAL*MAC1A= 1 CUM_TOT*MAC1A= C //OVERLAY;
→ TITLE WEEKLY (T) AND CUMULATIVE (C) PUPAE COUNTS BY MAC1B;
%
OPTIONS CENTER;
DATA OLD; INFILE BUG; INPUT
RT 2 SC $ 3-4 JD 17-18 BJD 16-18 YR 14-15 DATEA 14-18
METH $ 69 TOTAL 70-73 1;
WK=CEIL(RJD/7);
$ASDATE=DATEJUL(DATEA);
MON=MONTH($ASDATE);
QTR=CEIL(MON/4);
IF METH=1' : METH='S' : METH= A THEN METH= D';
IF METH=3 : METH='7' : METH='C' THEN METH= E ;
IF METH=2 : METH= 6 : METH='B' THEN METH= F';
IF MAC1C;
IF MAC1D<=DATEA<=MAC1E AND (MAC1F);
PROC SORT DATA=OLD; BY MAC1;
PROC MEANS NOPRINT MIN DATA=OLD; BY MAC1;
VARIABLES DATEA; OUTPUT OUT=TRY1T2 MIN=STARTDTE;
DATA OLD2;
MERGE TRY1T2 (IN=OK1) OLD (IN=OK2) ; BY MAC1;
IF OK1 & OK2;
PROC SORT DATA=OLD2; BY SC METH DATEA;
PROC MEANS NOPRINT MEAN STDERR SUM;
BY SC METH STARTDTE MAC1 NOTSORTED;
VARIABLES TOTAL ; OUTPUT OUT=GO1 MEAN=MEAN STDERR=S_E_ SUM=TOTAL;
PROC SORT; BY SC METH STARTDTE;
DATA READY; SET GO1; BY SC METH ;
RETAIN CUM_TOT 0 MAC1A 0;
IF FIRST.METH OR FIRST.SC THEN CUM_TOT=0;
IF FIRST.METH OR FIRST.SC THEN MAC1A=0;
CUM_TOT=CUM_TOT+TOTAL;
MAC1A=MAC1A+1;
PROC PRINT DATA=READY; FORMAT SC $SCC. METH $METH. MEAN S_E_ TOTAL 6.2 CUM_TOT 7.
2 ; BY SC METH; ID MAC1;
VARIABLES STARTDTE MAC1A MEAN S_E_ TOTAL CUM_TOT;
TITLE PUPAL PRODUCTION BY MAC1B;
DATA READY; SET READY;
SELECT=MAC1G;
IF SELECT=1;
MAC1H;
END OF DATA

```

```

exec d0101.stat 'p1'
FILE SORTLIB NOT FREED, IS NOT ALLOCATED
FILE SORTWK01 NOT FREED, IS NOT ALLOCATED
FILE SORTWK02 NOT FREED, IS NOT ALLOCATED
FILE SORTWK03 NOT FREED, IS NOT ALLOCATED
FILE SYSOUT NOT FREED, IS NOT ALLOCATED
NOTE: THE JOB SEANJ01 HAS BEEN RUN UNDER RELEASE 79.2B OF SAS AT WASHINGTON COMPUTER CENTER.
NOTE: THIS IS AN EXPERIMENTAL TEST RELEASE OF SAS. PLEASE
      REPORT ALL PROBLEMS TO YOUR INSTALLATION REPRESENTATIVE.

```

```

NOTE: INFILE BUG IS:
      DSN=SEANJ01.00101.TAD17.DATA,
      UNIT=SYSDA,VOL=SER=DATA18,DISP=SHR,
      DCB=(BLKSIZE=4840,LRECL=121,RECFM=FB)
NOTE: INFILE BUG HAS 5665 LINES.
NOTE: DATA SET WORK.OLD HAS 26 OBSERVATIONS AND 12 VARIABLES. 138 OBS/TRK.

NOTE: DATA SET WORK.OLD HAS 26 OBSERVATIONS AND 12 VARIABLES. 138 OBS/TRK.

NOTE: DATA SET WORK.TRYIT2 HAS 7 OBSERVATIONS AND 2 VARIABLES. 612 OBS/TRK.

NOTE: DATA SET WORK.OLD2 HAS 26 OBSERVATIONS AND 13 VARIABLES. 126 OBS/TRK.

NOTE: DATA SET WORK.OLD2 HAS 26 OBSERVATIONS AND 13 VARIABLES. 126 OBS/TRK.

NOTE: DATA SET WORK.G01 HAS 7 OBSERVATIONS AND 7 VARIABLES. 258 OBS/TRK.

NOTE: DATA SET WORK.G01 HAS 7 OBSERVATIONS AND 7 VARIABLES. 258 OBS/TRK.

NOTE: DATA SET WORK.READY HAS 7 OBSERVATIONS AND 9 VARIABLES. 192 OBS/TRK.

```

PUPAL PRODUCTION BY WEEK

14:00 WEDNESDAY, DECEMBER 26, 1979

1

----- SC=AA=CULICOIDES VARIIPENNIS SONORA METH=MLS OF PUPAE -----						
WK	STARTDTE	CUM_WK	MEAN	S_E	TOTAL	CUM_TOT
30	76205	1	4.75	0.75	19.00	19.00
31	76212	2	5.60	0.98	28.00	47.00
32	76219	3	4.50	0.65	18.00	65.00
33	76226	4	5.38	0.94	21.50	86.50
34	76233	5	3.13	0.83	12.50	99.00
35	76240	6	6.75	0.83	27.00	126.00
36	76247	7	7.00		7.00	133.00

```

NOTE: DATA SET WORK.READY HAS 7 OBSERVATIONS AND 10 VARIABLES. 168 OBS/TRK.

```

Figure 5.—SAS output from the pupal production program shown in fig. 4. The general data-retrieval request is shown in appendix C, 1.

A

LARVAL TEMPERATURE DATA BY WEEK

----- SC=AA=CULICOIDES VARIIPENNIS SONORA RM=3 -----									
WK	STARTDTE	MEAN_HI	SE_HI	HI_MAX	HI_MIN	MEAN_LO	SE_LO	LO_MAX	LO_MIN
30	76205	28.09	0.06	28.5	28.0	25.86	0.10	26.5	25.5
31	76211	28.47	0.08	29.0	28.0	25.20	0.10	26.0	25.0
32	76218	28.75	0.08	29.0	28.5	25.00	0.16	26.0	24.0
33	76225	28.08	0.10	28.5	27.5	25.00	0.20	26.0	24.0
34	76232	28.46	0.26	29.5	27.0	25.50	0.24	26.5	24.0
35	76239	28.25	0.14	29.0	27.5	26.08	0.22	27.0	25.0
36	76246	27.67	0.17	28.0	27.5	26.17	0.17	26.5	26.0

BTEMPERATURE DATA FOR LARVAL DEVELOPMENT
FOR SELECTED CAGES

----- SC=AA=CULICOIDES VARIIPENNIS SONORA DAYS=16 -----										
LCN	STARTDTE	END_DTE	MEAN_HI	SE_HI	HI_MAX	HI_MIN	MEAN_LO	SE_LO	LO_MAX	LO_MIN
2400	76155	76171	27.47	0.14	28.50	26.50	25.44	0.16	26.50	24.00
2401	76156	76172	27.47	0.14	28.50	26.50	25.47	0.15	26.50	24.00
2402	76156	76172	27.47	0.14	28.50	26.50	25.47	0.15	26.50	24.00
2403	76157	76173	27.47	0.14	28.50	26.50	25.56	0.14	26.50	24.00

Figure 6.—A, Output from retrieval request for larval rearing temperature by week (see appendix C, 3). B, Output from retrieval request for histories of larval rearing temperatures for specific cages of adult flies (see appendix C, 3).

A

SUMMARY OF ADULT DISPOSAL

BY WEEK

----- YR=76 SC=AA=CULICOIDES VARIIPENNIS SONORA -----

WK	STARTDTE	CAGES	COLONY	RESEARCH	BOTH	DISCARD
30	76205	4	0.00	0.00	4.00	0.00
31	76211	15	8.00	7.00	0.00	0.00
32	76218	2	0.00	0.00	2.00	0.00
33	76225	12	10.00	2.00	0.00	0.00
34	76232	12	12.00	0.00	0.00	0.00
35	76239	4	0.00	0.00	4.00	0.00
36	76246	3	2.00	1.00	0.00	0.00
TOTAL	.	52	32.00	10.00	10.00	0.00
% TOTAL	.	.	61.54	19.23	19.23	0.00

B

USE OF FLIES - LISTING BY WEEK

--- SC=AA=CULICOIDES VARIIPENNIS SONORA WK=30 STARTDTE=76205 END_DTE=76210 ---

CAGE	DISP1	DISP2
2461	ADULTS FOR BOTH	ADULTS FOR BOTH
2462	ADULTS FOR BOTH	ADULTS FOR BOTH
2463	.	ADULTS FOR COLONY
2464	ADULTS FOR RESEARCH	.
2465	.	ADULTS FOR COLONY
2466	ADULTS FOR RESEARCH	.
2467	.	ADULTS FOR COLONY
2468	ADULTS FOR RESEARCH	.
2469	.	ADULTS FOR COLONY
2470	ADULTS FOR RESEARCH	.
2471	.	ADULTS FOR COLONY

Figure 7.—A, Output from retrieval request for summary of adult fly use (disposal) by week (see appendix C, 8). B, Output from retrieval request for summary of adult fly use (disposal) for specific cages of adult flies (see appendix C, 8).

A

----- FEEDINGS=1 SC=AB=PSOROPHORA COLUMBIAE START=76205 END_DTE=76247 -----

DATE	CAGE	SEQUENCE	TYPE
76220	113	1	10% SUCROSE + WATER
76230	114	1	10% SUCROSE + WATER

----- FEEDINGS=3 SC=AA=CULICOIDES VARIIPENNIS SONORA START=76205 END_DTE=76247 -----

DATE	CAGE	SEQUENCE	TYPE
76207	2464	1	WATER ONLY
76208	.	2	10% SUCROSE + WATER
76208	.	3	WATER ONLY
76208	2466	1	WATER ONLY
76213	.	2	10% SUCROSE + WATER

B

JULIAN DATE OF Q.C. SAMPLE	NO. OF PUPAE PER ML.	MEAN WING LENGTH (MM) +OR- S.E.	MEAN DRY WEIGHT (MICROGRAMS) +OR- S.E.
76238	702	1.45 +OR- 0.01	83.43 +OR- 2.59
76244	687	1.48 +OR- 0.02	87.30 +OR- 2.90
76252	712	1.46 +OR- 0.01	84.07 +OR- 3.11
76257	672	1.51 +OR- 0.01	93.78 +OR- 2.80
76264	670	1.50 +OR- 0.01	93.30 +OR- 2.69
76271	686	1.47 +OR- 0.01	89.60 +OR- 2.24

Figure 8.—A, Outputs from retrieval requests for summary of nutritional regimes for a mosquito and a gnat colony (see appendix C, 10). B, Output from retrieval request for quality-control data on fly size for a specific colony and time span (see appendix C, 12).

pupal production program. Then data inputs were entered (for example, Julian dates,³ colony codes, and cage numbers—appendix C). Next, the SAS program was run and the report listed (printed) as output. The data inputs were made or changed after the MACRO MAC 1 X statements that appeared after each CHOOSE, PLACE, or SELECT statement. The output generated by running the SAS pupal production program (fig. 4) is shown in fig. 5 (plot excluded). Examples of the tables produced from some of the retrieval programs listed in appendix C are shown in figs. 6–8, with the execution statements and plots omitted for brevity. The actual computer operation of the retrieval programs and the system itself required several computer programs (appendix D).

Discussion

Since every insectary can benefit from logical flow charts and well-planned data-collection documents, systems analysis is advantageous even without being linked to a computer, as it encourages efficient performance of tasks. In particular, coding and recording of quantitative information increases the accuracy of data collection. Technicians find the ADP system easy to use and, because of the flow charts and clearly marked forms, are unlikely to forget duties or fail to record important information. The system is an effective management tool since consistent and easily reviewed documents are readily provided by the computer to evaluate variables associated with insect production—environmental conditions; nutritional regimens; and the quality, quantity, and use of insects. Therefore, one of our goals is to enter data into the computer at least weekly so that we can immediately change procedures in response to quality-control and production reports (see “Putting the Control in Quality Control in Insect Rearing,” by D. L. Chambers and T. R. Ashley and “The Closed-Loop System of Quality Control in Insect Rearing,” by J. C. Webb).

Our system has evolved slowly; by 1980, we had used the data-collection part for more than 4 years, the editing part for 2 years, and the retrieval request for 6 months. But the current data base is restricted to the one 80-column entry document. This limitation has caused some codes (for example, for pupal production) to become complex and some information to be lost (for example, disposition of flies coded for research without an indication of the recipient). So we are expanding the system to include almost every aspect of our insect-rearing program. The subjects eventually covered by this expansion

will include adult production, physical variables and their parameters, pupal and egg production, quality control, larval production and use of immatures, production costs, and shipments to other laboratories. We will also enter the data through the TSO terminal rather than from computer cards.

For those who might wish to use systems analysis but are unfamiliar with computer technology, we recommend an introductory computer course that will give them a working knowledge of the terminology. Then, a systems analyst could be consulted before a new system for a specific insect-rearing operation is planned or created. The application of systems analysis should include an assessment and examination of the organization and operation of the insect-rearing program, evaluation of existing data-collection forms and other documents, completion of a feasibility study determining the usefulness of ADP to the particular program, design, and implementation. After the system is in place, there should be an evaluation, and changes should be made to improve the system if necessary. The techniques required to accomplish these steps include flow charting, summarizing, coding, and form designing. Also, computer software and hardware needs would have to be considered. We encourage those who incorporate these concepts into insect-rearing programs and hope that this report will foster use of systems analysis and information-system theory in the management of insect rearing.

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³Julian date: a 5-digit system of recording dates that begins with the last 2 digits of the year followed by 3 digits to record the day in a numbering system between 1 and 365/6 for each year; Feb. 15, 1981, would be 81046.

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Appendix A.—Computer Codes and Editing Criteria for the Colonies-Adult-Production Record Document, Figure 3

Column number	Description
1-15	I. <i>Record identifier</i> .—Alphanumerically coded data are stored in 15 columns comprising 7 fields that identify each record (a horizontal line of data in figure 3) from all other records.
1	A. <i>File code</i> .—1-column numeric field to denote investigator or research project. Valid entry is 1 (entomology).
2	B. <i>Record type</i> .—1-column numeric field to denote type of research record used. Valid entry is 1 (colonies-adult-production record).
3-9	C. <i>Population</i> .
	1. <i>Colony</i> .—2-column, alphabetic field to record genera, species, and particular population of insects that pertain to data in the record. Valid entries for established colonies are AA-BZ. Code ZZ means no adult insects are present, and only location data in 53-68 are being recorded for the year and day given in 14-18; columns 5-13, 19-52, and 69-79 must be blank.
	Species
	AA— <i>Culicoides variipennis</i> (000) Texas 1957.
	AD— <i>Aedes aegypti</i> Texas 1974.
	AF— <i>Culex tarsalis</i> Colorado 1972.
	AG— <i>Culiseta inornata</i> Colorado 1972.
	AK— <i>Culicoides variipennis</i> (036) Idaho 1973.
	AP— <i>Aedes taeniorhynchus</i> Florida 1975.
5-8	2. <i>Large cage No.</i> —4-column numeric field that identifies large emergence cages. The number is assigned sequentially for each large cage when it is established. For each colony, numbers must follow sequentially and may not be duplicated unless each duplicate is accompanied by a different small-cage number in 9-11. Valid entries are 0001-9999.
9-11	3. <i>Small cage No.</i> —3-column numeric field that identifies small cages set up sequentially from 1 large cage. Numbers may not be duplicated if they are accompanied by an identical large-cage number in 5-8. Small-cage numbers are usually issued by the user of flies so that field 9-11 is usually blank. Valid entries are 001-999.
12-13	D. <i>Generation</i> .—2-column numeric field to denote generation of colony. Parent generation (P) is 01. The number is sequential for successive generations. If the colony is a population without discrete generations, the code is 00. The same generation number is valid for multiple records with the same colony code, but nonsequential generation numbers are invalid (the generation number must be the same or in sequence). Valid entries are 00-99.
14-15	E. <i>Year</i> .—2-column numeric field that denotes the year that data were collected. The last 2 digits of the year are entered. Valid entries are less than or equal to the current year and greater than or equal to 74.
16-28	II. <i>Emergence time</i> .—Numerically coded data are stored in 13 columns comprising 4 fields to record start and end of insect emergence.
16-22	A. <i>Start</i> . (Pupae placed in cage).
	1. <i>Julian day</i> .—3-column numeric field that denotes Julian day. Valid entries are 001-365 for regular years and 001-366 for leap years.
19-22	2. <i>Time</i> .—4-column numeric field to record time of day when pupae are placed in the cage. Military time is used. Laboratory practice is to record to the closest hour; for some special tests, the entry may be to the nearest minute. Valid entries are 0001-2400.
23-28	B. <i>End</i> . (Pupae removed from cage).
23-24	1. <i>Day</i> .—2-column numeric field to record last 2 digits of Julian day. Entries must be greater than or equal to the Julian day in 16-18, and elapsed days from Julian day in 16-18 must be less than or equal to 14. Valid entries are 00-99.
25-28	2. <i>Time</i> .—4-column numeric field to record the time when pupae are removed from the cage. (See IIA2.) Valid entries are 0001-2400.

Column number	Description
29-52	III. <i>Adult nutrition</i> .—Numerically coded data are stored in 24 columns comprising 9 fields to record adult-nutrition data. A new feeding regimen begins when different nutrition is given to a cage of flies, and the code for food type must change.
29-36	A. <i>1st Feeding</i> .
29-30	1. <i>Day</i> .—2-column numeric field to record the last 2 digits of the Julian day on which the 1st feeding regimen was begun. Entries must equal the last 2 digits of the Julian day in 16-18. Valid entries are 00-99.
31-34	2. <i>Time placed</i> .—4-column numeric field to record the time the 1st feeding was begun. (See IIA2.) Valid entries are 0001-2400.
35-36	3. <i>Food type</i> .—2-column numeric field to record the first type of nutrition given. Water is always present for emerging adults in the pupal dish; so the 1st-feeding regimen is coded 01 if no other nutrition was given, and values in 16-22 are used for day and time. Valid entries are 01-05.

Codes

- 01—water only,
- 02—sucrose solution (5%–10%) with water given also,
- 03—sugar cubes and water,
- 04—raisins and water,
- 05—sugar solution for 1 day alternated with water only for 2 days.

37-44	B. <i>2nd Feeding</i> .
37-38	1. <i>Day</i> .—2-column numeric field to record the last two digits of the Julian day on which the 2d feeding regimen was begun. Columns 37-38 are blank if 29-36 are blank. The value in 37-38 must be greater than or equal to the value for 29-30. If the value in 29-30 equals that for 37-38, then the value in 31-34 must be less than the value in 39-42. Valid entries are 00-99.
39-42	2. <i>Time placed</i> .—4-column numeric field to record the time the 2d feeding regimen was begun. (See IIA2.) If the value in 37-38 equals that for 29-30, then the value in 39-42 must be greater than the value in 31-34. Valid entries are 0001-2400.
43-44	3. <i>Food</i> .—2-column numeric field to record the 2d type of nutrition given. (See IIIA3.) Columns 43-44 must be blank or contain a value different from that in 35-36. Valid entries are 01-05.
45-52	C. <i>3rd Feeding</i> .—If 37-44 are blank, then 45-52 must be blank.
45-46	1. <i>Day</i> .—2-column numeric field to record last 2 digits of the Julian day on which 3d feeding regimen was begun. Columns 45-46 are blank if 37-44 are blank. The value in 45-46 must be greater than or equal to the value in 37-38. If the value in 37-38 equals that for 45-46, then the value in 39-42 must be less than the value in 47-50. Valid entries are 00-99.
47-50	2. <i>Time placed</i> .—4-column numeric field to record the time 3d feeding regimen was begun (See IIA2.) If the value in 45-46 equals that for 37-38, then the value in 47-50 must be greater than the value in 39-42. Valid entries are 0001-2400.
51-52	3. <i>Food type</i> .—2-column numeric field to record the 3d type of nutrition given. (See IIIA3.) Columns 51-52 must be blank or have a value different from that in 43-44. Valid entries are 01-05.
53-68	IV. <i>Location</i> .—Numerically coded data are stored in 16 columns comprising 7 fields to record data on physical variables (see "physical parameters" in fig. 3) for insect-rearing rooms for the Julian day entered in columns 16-18. The 7 location data fields, 53-68, may not be blank if the serial code in 3-4 is ZZ.
53-54	A. <i>Room</i> .—2-column numeric field to code the room in the building where environmental data are taken. Valid entries are 01-09.

Codes

- 01—building 106-B, colony room 1,
- 02—building 106-B, colony room 2,
- 03—building 106, colony room,
- 04—building 106-A, colony room,
- 09—building 106, laboratory.

Column number	Description
55-68	B. <i>Physical parameters.</i> —Variables in a room or incubator for a given day.
55-60	
55-57	
58-60	
61-68	
61-64	
61-62	
63-64	
65-68	
65-66	
67-68	
69-73	V. <i>Pupal Prod.</i> —Alphanumerically coded data are stored in 5 columns comprising 2 fields to record the counting method and the number of pupae collected.
69	A. <i>CM.</i> —Counting method; one-column alphanumeric field to record method used for pupal counts in 70-73. The numeric code records pupae picked (harvested) on the same date (same batch). The alphabetic code records pupae combined from different picking dates. Valid entries are 1-7 and A-C.

Codes

- 1—number of pupae per milliliter;
- 2—estimate;
- 3—actual count;
- 4—pupae picked previously and remaining pupae moved to new cage;
- 5—pupae picked, placed in more than 1 cage, and all cages labeled "5;" the value equals the number of pupae per milliliter;
- 6—pupae picked, placed in more than 1 cage, and all cages labeled "6;" the value is estimated;
- 7—pupae picked, placed in more than 1 cage, and all cages labeled "7;" the value equals the actual count.
- A—pupae, from different picking dates, combined; the value equals the number of pupae per milliliter;
- B—pupae, from different picking dates, combined; the value is estimated;
- C—pupae, from different picking dates, combined; the value equals the actual count.

70-73	B. <i>No.</i> —4-column numeric field to record the number or amount of pupae. If codes that record the number of pupae per milliliter in column 69 are 1, 5, or A, a decimal point is assumed between 72-73. If the value for 70-73 is 0000, then the code for the counting method in column 69 must be 4, A, B, or C. If column 69 has an alphabetic code, then 0000 in 70-73 means pupae are recorded on a trailer card (a second card); the value in 70-73 (with an alphabetic code in column 69) records only new pupae that were placed with the remaining older pupae. Valid entries are 0000-9999.
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Column number	Description
74-79	VI. <i>Adult disposition</i> .—Numerically coded data are stored in 6 columns comprising 4 fields to record whether flies were used for research and/or for a colony; 2 divisions of adult disposition—research and colony—contain 2 fields each to record how and when flies were used.
74-76	A. <i>Research</i> .
74	1. <i>How</i> .—1-column numeric field to record each large cage of flies that was used for research. If the value in column 74 is 3, then the value in column 77 must be 3. If column 74 is blank, then the value in column 77 must be 1 or 5. The values 1 and 5 may only be used in column 77; 3 may be used in both columns 74 and 77. Valid entries are blank, 2 or 3.
	Codes
	1—adults to colony, 2—adults to research, 3—adults to both, 4—undefined, 5—discarded.
75-76	2. <i>Day</i> .—2-column numeric field to record the last 2 digits of the Julian day when flies were used for research. Values must be greater than or equal to the Julian day in 16-18 and less than or equal to the value in 78-79. Valid entries are 00-99.
77-79	B. <i>Colony</i> .
77	1. <i>How</i> .—1-column numeric field to record large cage of flies used for a colony. (See VIA1.) If the value in column 77 is 3, then the value in column 74 must be 3. If column 77 is blank, then the value in column 74 must be 2. Valid entries are blank, 1, 3, and 5.
78-79	2. <i>Day</i> .—2-column numeric field to record last 2 digits of the Julian day when flies were used for the colony. If the value in column 77 is 3, then the value in 78-79 must be greater than or equal to that in 75-76. Valid entries are 00-99.
80	VII. <i>Card type</i> .—Numerically coded data are stored in a 1-column field to distinguish the primary card from trailer cards. The field may not be blank. Valid entry is 1 for the primary card.

Appendix B.—Computer Program Written From the Editing Document, Appendix A, to Verify Data⁴

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LIST 'SEANJ01.D0101.COLVERIF.CODOL'
'BEANJ01.D0101.COLVERIF.CODOL'
00010 IDENTIFICATION DIVISION.
00020 PROGRAM-TO. COLVERIF.
00030 DATE-WRITTEN. DECEMBER, 1977.
00040 ENVIRONMENT DIVISION.
00050 CONFIGURATION SECTION.
00060 SPECIAL-NAMES.
00070 CBI IS TOPLINE
00080 CBI IS HOME.
00090 SOURCE-COMPUTED. IBM-370.
00100 OBJECT-COMPUTED. IBM-370.
00110 INPUT-OUTPUT SECTION.
00120 FILE-CONTROL.
00130 SELECT TRANH ASSIGN TO UT-8-INT.
00140 SELECT MASTR ASSIGN TO UT-8-OUT1.
00150 SELECT ELIST ASSIGN TO UT-8-OUT2.
00160 SELECT ERHOB ASSIGN TO UT-8-INT.
00170 DATA DIVISION.
00180 FILE SECTION.
00190 *
00200 * TRANH IS THE INPUT TRANSACTION FILE.
00210 *
00220 FB TRANH
00230 RECORDING MODE IS F
00240 LABEL RECORDS ARE STANDARD
00250 BLOCK CONTAINS 0 RECORDS
00260 DATA RECORD IS IMPT.
00270 01 IMPT PIC X(121).
00280 *
00290 * MASTR IS THE OUTPUT FILE. ALL RECORDS THAT ARE ERROR FREE ARE
00300 * WRITTEN TO THIS FILE. THIS FILE CONTAINS ONLY DATA FROM THIS
00310 * PASS.
00320 *
00330 FB MASTR
00340 RECORDING MODE IS F
00350 LABEL RECORDS ARE STANDARD
00360 BLOCK CONTAINS 0 RECORDS
00370 DATA RECORD IS OUT.
00380 01 OUT PIC X(121).
00390 *
00400 * LIST IS THE PRINTED TRANSACTION/ERROR LIST.
00410 *
00420 FB ELIST
00430 RECORDING MODE IS F
00440 LABEL RECORDS ARE STANDARD
00450 BLOCK CONTAINS 0 RECORDS
00460 DATA RECORD IS PRT.
00470 01 PRT.
00480 03 FILLER PIC X.
00490 03 DORT PIC X(121).
00500 03 FILLER PIC X(11).
00510 *
00520 * ERHOB IS THE FILE OF ERROR MESSAGES. EACH ERROR MESSAGE HAS A
00530 * SEQUENTIAL NUMERIC CODE IN OC 79-80. ERROR MESSAGES CAN BE
00540 * ADDED AS NEEDED, INCREASING THE SUBSCRIPT AND INCREASING THE
00550 * VALUE IN STATEMENTS # 277 TO REFLECT THE INCREASED SIZE
00560 * ( INCREMENTS OF 80 ), STMT # 278 'OCCURS X TIMES' ( INCREMENTS
00570 * OF 1 ), STMT # 289 ( INCREMENTS OF 1 ) AND STMT # 290 'OCCURS
00580 * X TIMES' ( INCREMENTS OF 1 ). SINCE EACH MESSAGE CARRIES ITS OWN
00590 * SUBSCRIPT VALUE, THESE RECORDS DO NOT HAVE TO BE IN SEQUENCE
00600 * WHEN THEY ARE READ IN.
00610 *
00620 FB ERHOB
00630 RECORDING MODE IS F
00640 LABEL RECORDS ARE STANDARD
00650 BLOCK CONTAINS 0 RECORDS
00660 DATA RECORD IS E-M.
00670 01 E-M.
00680 03 FILLER PIC X(78).
00690 03 E-MO PIC 99.
00700 WORKING-STORAGE SECTION.
00710 *
00720 * A IS INCREMENTED IN STMT # 440, AND IS USED TO CALCULATE THE DAYS
00730 * UNTIL EMERGENCE.
00740 *
00750 77 A PIC 999 VALUE 000.
00760 77 LINEI PIC 99 VALUE 99.
00770 *
00780 * DAT-STORE RECEIVES THE FIRST FEEDING DAT FOR NUMERIC COMPARISONS.
00790 *
00800 77 DAT-STORE PIC 99 VALUE 00.
00810 *
00820 * B IS INCREMENTED IN THE ERROR CHECK LOOP (STMT # 707).
00830 *
00840 77 B PIC 99 VALUE 00.
00850 77 1ST PIC 9 VALUE 0.
00860 77 NROD PIC 9(5) VALUE 00000.
00870 77 NROTE PIC 9(5) VALUE 00000.
00880 77 NERR PIC 9(5) VALUE 00000.
00890 77 NO-SU PIC X VALUE ' '.
00900 01 P10JN PIC X(11) VALUE HIGH-VALUES.
00910 *
00920 * DUPE51 AND DUPE52 ARE USED TO CHECK FOR 80 COLUMN DUPLICATES IN
00930 * THE TRANSACTION FILE.
00940 *
00950 01 DUPE51.
00960 03 DUPEH01 PIC X(121).
00970 03 FILLER REDEFINES DUPEH01.
00980 05 RECH01 PIC X(114).
00990 05 FILLED PIC X(7).
01000 01 DUPE52.
01010 03 DUPEH02 PIC X(121).
01020 03 FILLER REDEFINES DUPEH02.
01030 05 RECH02 PIC X(114).
01040 05 FILLED PIC X(7).
01050 *
01060 * J-BAT-CALC IS USED IN EMERGENCE CALCULATIONS AS WELL AS INTER-DATE
01070 * RELATIONSHIPS.
01080 *
01090 01 J-BAT-CALC.
01100 03 J-BAT-DATE PIC 999.
01110 03 FILLER REDEFINES J-BAT-DATE.
01120 05 FILLED PIC 9.
01130 05 RESULT PIC 99.
01140 01 FILLER.
01150 03 EOB PIC X VALUE ' '.
01160 05 END-OF-DATA VALUE 'X'.
01170 *
01180 * BASEOTE-BRN BREAKS DOWN THE DATE JULIAN DAT 80 THE LAST 2 81BITS
01190 * ARE AVAILABLE FOR COMPARISONS.
01200 *
01210 01 BASEOTE-BRN.
01220 03 HOLD PIC 999.
01230 03 BREAK REDEFINES H019.
01240 05 FILLED PIC 9.
01250 05 LAST-2 PIC 99.
01260 05 LST-2 REDEFINES LAST-2 PIC XX.
01270 *
01280 * WORK-REC RECEIVES THE TRANSACTIONS ONE AT A TIME.
01290 *
01300 01 WORK-REC.
01310 03 ID1.
01320 05 FILE-TYPE PIC X.
01330 05 FILE-OK VALUES '1'. PIC X.
01340 05 RECORD-TYPE PIC X.
01350 05 RECORD-OK VALUES '1'.
01360 05 SERIAL-CODE PIC XX.
01370 05 SER-CO-OK VALUES 'AA' THRU 'D2' '12'.
01380 05 AK-COLORIT VALUE 'AK'.
01390 05 12-COLORIT VALUE '12'.
01400 05 LB-CAGE PIC XXXX.
01410 05 LABE-CAGE-OK VALUES '0001' THRU '9999'.
01420 05 12-CAGE VALUE ' '.
01430 05 SH-CAGE PIC XXX.
01440 05 SMALL-CAGE-SK VALUES '000' THRU '999' ' '.
01450 03 GENERATION PIC XX.
01460 05 GENERATION-OK VALUES '01' THRU '99' ' '.
01470 05 AA-GENERATION VALUE '00'.
01480 02 J0.
01490 03 BADE-DATE.
01500 05 B-TEAR PIC XX.
01510 05 TEAR-VALID VALUES '74' THRU '99'.
01520 05 LEAP-TEAR VALUES '74' '80' '84' '88' '92' '96'.
01530 05 B-BAT PIC XXX.
01540 05 BAY-VAL10 VALUES '001' THRU '345'.
01550 05 BAY-366 VALUE '366'.
01560 03 EMERGENCE-0-TIME PIC XXXX.
01570 05 START-TIME-OK VALUES '0001' THRU '2400'.
01580 05 AK-0T01-TIME VALUE '0001' THRU '2400'.
01590 03 EMERGENCE-END-DAT PIC XX.
01600 05 VAL10-END-DAT VALUES '00' THRU '99'.
01610 03 ENDS-END REDEFINES EMERGENCE-END-DAT PIC 99.
01620 02 FILLED.
01630 03 EMERGENCE-E-TIME PIC XXXX.
01640 05 ERO-TIME-OK VALUES '0001' THRU '2400'.
01650 05 AK-ENO-TIME VALUE '0001' THRU '2400'.
01660 03 FEED1-DAT PIC XX.
01670 05 VAL10-FEED1-DAT VALUES '00' THRU '99' ' '.
01680 03 F1-CHK REDEFINES FEED1-DAT PIC 99.
01690 03 FEED1-TIME PIC XXXX.
01700 05 VAL10-1ST-FEED-TIME VALUES '0001' THRU '2400' ' '.
01710 05 AK-1ST-FEED-TIME VALUE '0001' THRU '2400' ' '.
01720 03 TYPE-FB001 PIC XX.
01730 05 VAL10-FB001-TYPE VALUES '01' THRU '05' ' '.
01740 03 FEED2-DAT PIC XX.
01750 05 VAL10-FEED2-DAT VALUES '00' THRU '99' ' '.
01760 05 NO-2ND-FEED-DAT VALUES ' ' '0000'.
01770 03 FEED2-TIME PIC XXXX.
01780 05 VAL10-2ND-FEED-TIME VALUES '0001' THRU '2400' ' '.
01790 05 AK-2ND-FEED-TIME VALUE '0001' THRU '2400' ' '.
01800 03 TYPE-FB002 PIC XX.
01810 05 VAL10-FB002-TYPE VALUES '01' THRU '05' ' '.
01820 03 FEED3-DAT PIC XX.
01830 05 VAL10-FEED3-DAT VALUE '00' THRU '99' ' '.
01840 05 NO-3RD-FEED-DAT VALUES ' ' '0000'.
01850 03 FEED3-TIME PIC XXXX.
01860 05 VAL10-3RD-FEED-TIME VALUES '0001' THRU '2400' ' '.
01870 05 AK-3RD-FEED-TIME VALUES '0000' THRU '2400' ' '.
01880 05 NO-3RD-FEED-TIME VALUES ' ' '00000'.
01890 03 TYPE-FB003 PIC XX.
01900 05 VAL10-FB003-TYPE VALUES '01' THRU '05'.
01910 03 F000-NO PIC XX.
01920 05 VAL10-F000-NO VALUES '01' THRU '09'.
01930 03 LARVAL-HIGH-TEMP PIC XXX.
01940 05 VAL10-LARVAL-HIGH VALUES '150' THRU '400' ' '.
01950 03 LARVAL-LOW-TEMP PIC XXX.
01960 05 VAL10-LARVAL-LOW VALUES '150' THRU '400' ' '.
01970 03 ADULT-HIGH-TEMP PIC XX.
01980 05 8000-HIGH-TEMP VALUES '15' THRU '40' ' '.
01990

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⁴Program based on earlier and slightly different versions of record and editing documents.

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02000 03 ADULT-LOW-TEMP PIC XX.
02010 00 BOOB-LOW-TEMP VALUES '15' THRU '40' ' '.
02020 03 ADULT-HIGH-RH PIC XX.
02030 00 VALID-HIGH-RH VALUES '00' THRU '99' ' '.
02040 03 ADULT-LOW-RH PIC XX.
02050 00 VALID-LOW-RH VALUES '00' THRU '99' ' '.
02060 03 METHOD-AND-NO PIC XXXXX.
02070 00 80TH-ZEROS VALUE '00000'.
02080 03 FILLER REDEFINES METHOD-AND-NO.
02090 05 COUNTNO-METHOD PIC X.
02100 00 VALID-C-METHOD VALUES 'A' THRU 'C' '1' THRU '7'.
02110 00 METHOD-NO-FLIES VALUES 'A' THRU 'C' '4'.
02120 05 NO-OF-FLIES PIC XXXX.
02130 06 ACCEPTABLE-RANGE VALUES '0000' THRU '9999'.
02140 06 NO-FLIES VALUE '0000'.
02150 03 DISPOSITION1 PIC X.
02160 00 DISP1-VALID VALUES '1' '2' '3'.
02170 00 ADULTS-TO-BOTH1 VALUE '3'.
02180 00 BOOB-W-BLANK VALUE '2'.
02190 03 DISP1-DAT PIC XX.
02200 00 VALID-DISP1 VALUES '00' THRU '99' ' '.
02210 03 DISPOSITION2 PIC X.
02220 00 DISP2-VALID VALUES '1' '3' '5'.
02230 00 ADULTS-TO-BOTH2 VALUE '3' '5'.
02240 03 BLANK-VALUE VALUE ' '.
02250 03 DISP2-DAT PIC XX.
02260 00 VALID-DISP2 VALUES '00' THRU '99' ' '.
02270 03 CARD-TYPE PIC X.
02280 00 CARD-TYPE-OK VALUE '1'.
02290 03 QUALITY-CONTROL-DATA PIC X(34).
02300 03 FORM-TYPE PIC X.
02310 03 BATCH PIC X(6).
02320 *
02330 * HEAD1 THRU HEAD6 ARE HEADINGS FOR THE ERROR LIST.
02340 *
02350 01 HEAD1.
02360 03 FILLER PIC X(44) VALUE
02370 ' AGRICULTURAL RESEARCH'.
02380 03 FILLER PIC X(36) VALUE
02390 ' H SERVICE'.
02400 03 FILLER PIC X(52) VALUE SPACES.
02410 01 HEAD2.
02420 03 FILLER PIC X(44) VALUE
02430 ' ARTHROPOD-BORNE ANIMAL DISEASE'.
02440 03 FILLER PIC X(36) VALUE
02450 ' RESEARCH LABORATORY'.
02460 03 FILLER PIC X(52) VALUE SPACES.
02470 01 HEAD3.
02480 03 FILLER PIC X(44) VALUE
02490 ' DENVER FEDERAL CENTER - B'.
02500 03 FILLER PIC X(20) VALUE
02510 ' ENVER, COLORADO'.
02520 03 MM PIC XX.
02530 03 FILLER PIC X VALUE '/''.
02540 03 DB PIC XX.
02550 03 FILLER PIC X VALUE '/''.
02560 03 YI PIC XX.
02570 03 FILLER PIC X(52) VALUE SPACES.
02580 01 HEAD4.
02590 03 FILLER PIC X(44) VALUE
02600 ' TRANSACTION / ERROR'.
02610 03 FILLER PIC X(36) VALUE
02620 ' LISTING'.
02630 03 FILLER PIC X(52) VALUE SPACES.
02640 01 HEAD5.
02650 03 FILLER PIC X(44) VALUE
02660 ' 1 2 3 4 '.
02670 03 FILLER PIC X(36) VALUE
02680 ' 5 6 7 8 '.
02690 03 FILLER PIC X(52) VALUE SPACES.
02700 01 HEAD6.
02710 03 FILLER PIC X(44) VALUE
02720 ' 12345678901234567890123456789012345678901234'.
02730 03 FILLER PIC X(36) VALUE
02740 ' 567890123456789012345678901234567890'.
02750 03 FILLER PIC X(52) VALUE SPACES.
02760 *
02770 * ERROR-TABLE IS THE RECEIVING AREA FOR THE ERROR MESSAGES.
02780 *
02790 01 ERROR-TABLE.
02800 03 ERR-TBL PIC X(4320).
02810 03 E-TBL REDEFINES ERR-TBL OCCURS 54 TIMES.
02820 05 E-M PIC X(78).
02830 05 E-M PIC XX.
02840 *
02850 * ERROR-KEY HAS A 1 CHARACTER FLAG FOR EACH ERROR MESSAGE. AS AN
02860 * ERROR IS DETECTED, AN 'X' IS MOVED TO E-KEY (XX) WHERE XX IS
02870 * THE ERROR MESSAGE SUBSCRIPT. THEN, AS THE ERROR ROUTINE PROGRESSES
02880 * IT PRINTS THE MESSAGE CORRESPONDING TO THE RELATIVE POSITION
02890 * WHERE AN 'X' IS FOUND.
02900 *
02910 01 ERROR-KEY.
02920 03 ERR-KEY PIC X(54).
02930 03 E-KEY REDEFINES ERR-KEY PIC X OCCURS 54 TIMES.
02940 *
02950 * U-LINE HAS DATA ELEMENTS THAT CORRESPOND TO DATA ELEMENTS IN THE
02960 * TRANSACTION RECORD. WHEN A FIELD IS FOUND TO BE IN ERROR, ' '
02970 * (UNDERScores) ARE MOVED TO THE AREA CORRESPONDING TO THE FIELD
02980 * IN ERROR AND PRINTED AFTER THE RECORD SUPPRESSING LINE FEED TO
02990 * UNDERLINE THE DATA ELEMENT IN ERROR.
03000 *
03010 01 U-LINE.
03020 03 FT PIC X.
03030 03 RT PIC X.
03040 03 SC PIC XX.
03050 03 LC PIC XXXX.
03060 03 SMC PIC XXX.
03070 03 SM PIC XX.
03080 03 BI PIC XX.
03090 03 BD PIC XXX.
03100 03 ES PIC XXXX.
03110 03 EED PIC XX.
03120 03 EET PIC XXXX.
03130 03 F1D PIC XX.
03140 03 F1T PIC XXXX.
03150 03 TF1 PIC XX.
03160 03 F2D PIC XX.
03170 03 F2T PIC XXXX.
03180 03 TF2 PIC XX.
03190 03 F3D PIC XX.
03200 03 F3T PIC XXXX.
03210 03 TF3 PIC XX.
03220 03 RN PIC XX.
03230 03 LMT PIC XXX.
03240 03 LLT PIC XXXX.
03250 03 AMT PIC XX.
03260 03 ALT PIC XX.
03270 03 AMR PIC XX.
03280 03 ALR PIC XX.
03290 03 CM PIC X.
03300 03 MF PIC XXXX.
03310 03 B1 PIC X.
03320 03 D1D PIC XX.
03330 03 D2 PIC X.
03340 03 D2O PIC XX.
03350 03 CT PIC X.
03360 01 GET-DATE.
03370 02 FILLER PIC X(6).
03380 02 THIS-YEAR PIC XX.
03390 PROCEDURE DIVISION.
03400 INITIALIZE.
03410 DPM INPUT TRANS ERMSG OUTPUT MASTR ELIST.
03420 MOVE ZEROS TO J-DAY-BASE ERROR-KEY.
03430 MOVE SPACES TO WDRK-REC ERROR-TABLE U-LINE.
03440 PERFORM ERROR-LOAD UNTIL END-OF-DATA.
03450 MOVE CURRENT-DATE TO GET-DATE.
03460 GO TO STARTT.
03470 *
03480 * ERRDR-LOAD READS THE DECK OF ERRDR MESSAGES AND LOADS THEM INTO
03490 * ERROR-TABLE (CC79-80).
03500 *
03510 ERRDR-LOAD.
03520 READ ERMSG AT END MOVE 'X' TO EDD.
03530 MOVE ER TO E-TBL (E-NO).
03540 *
03550 * STARTT IS THE DRIVER OF THE PRDDRAW.
03560 *
03570 STARTT.
03580 MOVE ' ' TO EDD.
03590 PERFORM MAIN THRU MAIN-EXIT UNTIL END-OF-DATA.
03600 DISPLAY 'MASTER RECORDS READ' NRED.
03610 DISPLAY 'MASTER RECORDS WRITTEN' NROTE.
03620 DISPLAY 'MASTER RECORDS WITH ERRORS' NERR.
03630 CLOSE TRANS MASTR ELIST ERMSG.
03640 STOP RUN.
03650 *
03660 * MAIN IS THE BEGINNING OF THE MAJOR PERFORM STATEMENT. THE EDIT OF
03670 * EACH TRANSACTION RECORD STARTS HERE AND PROGRESSES THROUGH
03680 * MAIN-EXIT (STMT N 766). IT CONTAINS INDIVIDUAL FIELD VALIDITY
03690 * CHECKS, INTER-FIELD RELATIONAL EDITS AS WELL AS ERROR CHECK
03700 * AND PRINT ROUTINES.
03710 *
03720 MAIN.
03730 READ TRANS INTO WORK-REC AT END MOVE 'X' TO EDD.
03740 IF END-OF-DATA GO TO MAIN-EXIT.
03750 ADD 1 TO NREQ.
03760 IF ZZ-COLONY GO TO FILE-CK.
03770 IF T01 = PIDJM MOVE 'X' TO E-KEY (54). MOVE I01 TO PIDJM. 7/78 JDM
03780 MOVE WDRK-REC TO DUPEHOLD1.
03790 IF RECHOLD1 NOT = RECHOLD2 MOVE DUPEHOLD1 TO DUPEHOLD2
03800 ELSE MOVE 'X' TO E-KEY (54) MOVE DUPEHOLD1 TO
03810 DUPEHOLD2.
03820 *
03830 * FILE-CK VALIDATES CC 1.
03840 *
03850 FILE-CK.
03860 IF FILE-OK GO TO RECDR-CK ELSE MOVE 'X' TO E-KEY (01) MOVE
03870 ' ' TO FT.
03880 *
03890 * RECDR-CK VALIDATES CC 2.
03900 *
03910 RECDR-CK.
03920 IF RECDR-OK GO TO SER-CK ELSE MOVE 'X' TO E-KEY (02) MOVE
03930 ' ' TO RT.
03940 *
03950 * SER-CK VALIDATES CC 3-4.
03960 *
03970 SER-CK.
03980 IF SERIAL-CODE = ZZ PERFORM ZZ-CHECK THRU ZZ-EXIT GO TO
03990 REC-PRT.
04000 IF SER-CK-OK GO TO LRG-CGE-CK ELSE MOVE 'X' TO E-KEY (03)
04010 MOVE ALL ' ' TO SC.
04020 *
04030 * LRG-CGE-CK VALIDATES CC 5-8.

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04040 *
04050 LRS-CGE-CK.
04060 IF SERIAL-CODE = 'ZZ' AND ZZ-CAGE GO TO SM-CGE-CK.
04070 IF LARGE-CAGE-OK GO TO SM-CGE-CK ELSE MOVE 'X' TO E-KEY (04)
04080 MOVE ALL ' ' TO LC.
04090 *
04100 * SM-CGE-CK VALIDATES CC 9-11.
04110 *
04120 SM-CGE-CK.
04130 IF SMALL-CAGE-OK NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (05)
04140 MOVE ALL ' ' TO SMC.
04150 *
04160 * GENERATION-CK VALIDATES CC 12-13.
04170 *
04180 GENERATION-CK.
04190 IF SERIAL-CODE = 'AA' AND AA-GENERATION GO TO B-DATE-CK.
04200 IF GENERATION-OK NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (06)
04210 MOVE ALL ' ' TO GM.
04220 *
04230 * B-DATE-CK VALIDATES BAGE DATE CC 14-18.
04240 *
04250 B-DATE-CK.
04260 IF DAY-366 AND LEAP-TEAR GO TO EMER-S-CK.
04270 IF YEAR-VALID NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (07) MOVE
04280 ALL ' ' TO D1.
04290 IF B-DATE > THIS-YEAR MOVE 'X' TO E-KEY (07) MOVE
04300 ALL ' ' TO D1.
04310 IF DAY-VALID NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (08) MOVE
04320 ALL ' ' TO D0.
04330 *
04340 * EMER-S-CK VALIDATES CC 19-22.
04350 *
04360 EMER-S-CK.
04370 IF AK-COLONY AND AK-START-TIME GO TO EMER-E-CK.
04380 IF START-TIME-OK NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (09)
04390 MOVE ALL ' ' TO ES.
04400 IF JO NOT NUMERIC MOVE 'X' TO E-KEY (09) MOVE ALL ' ' TO ES.
04410 *
04420 * EMER-E-CK VALIDATES CC 23-24 AND PERFORMS CALCULATIONS ON DATE
04430 * TILL EMERGENCE.
04440 *
04450 EMER-E-CK.
04460 MOVE B-DATE TO J-DAY-BASE.
04470 IF VALID-EMO-DATE NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (10)
04480 MOVE ALL ' ' TO EED GO TO E-T-CK.
04490 * TIME FOLLOWING 5 LINES WERE ADDED ON 2/28/79 AFTER THE PROGRAM
04500 * ADDED AFTER FINDING A BLANK IN THE EMER-ENO FIELD
04510 IF EMER-ENO NOT NUMERIC
04520 DISPLAY WORK-REC
04530 DISPLAY ' '
04540 DISPLAY ' * EMERGENCE END DAY (23-24) ILLEGAL AS RECORDED'
04550 GO TO STARTT.
04560 *
04570 * PERFORM VALIDATE VARYING A FROM 1 BY 1 UNTIL RESULT =
04580 * EMER-ENO.
04590 IF A < 15 GO TO E-T-CK ELSE MOVE 'X' TO
04600 E-KEY (11) MOVE ALL ' ' TO EEO GO TO E-T-CK.
04610 *
04620 * VALIDATE IS THE PERFORMED COMPUTATION OF DAYS TILL EMERGENCE.
04630 *
04640 VALIDATE.
04650 ADD 1 TO J-DAY-BASE.
04660 IF LEAP-YEAR AND J-DAY-BASE > 366
04670 COMPUTE J-DAY-BASE = J-DAY-BASE - 366 ELSE
04680 IF J-DAY-BASE > 365
04690 COMPUTE J-DAY-BASE = J-DAY-BASE - 365.
04700 *
04710 * E-T-CK VALIDATES CC 25-28.
04720 *
04730 E-T-CK.
04740 IF AK-COLONY AND AK-EMB-TIME GO TO FEED1-B-CK.
04750 IF EMB-TIME-OK NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (12) MOVE
04760 ALL ' ' TO EET.
04770 *
04780 * FEED1-B-CK VALIDATES CC 29-30, AND ITS RELATIONSHIP TO THE BASE DATE.
04790 *
04800 FEED1-B-CK.
04810 IF VALID-FEED1-DAY NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (16)
04820 MOVE ALL ' ' TO F13 GO TO FEED1-T-CK.
04830 MOVE FEED1-DAY TO J-DAY-BASE.
04840 MOVE B-DAY-BASE TO J-DAY-BASE.
04850 IF DAY-BASE < RESULT MOVE 'X' TO E-KEY (13) MOVE ALL ' '
04860 TO F10.
04870 MOVE B-DAY-BASE TO HOLD.
04880 IF FEED1-DAY NOT = LST-2 MOVE 'X' TO E-KEY (14) MOVE ALL ' '
04890 TO F10.
04900 *
04910 * FEED1-T-CK VALIDATES CC 31-34.
04920 *
04930 FEED1-T-CK.
04940 IF AK-COLONY AND AK-1ST-FEED-TIME GO TO FEED1-TYPE-CK.
04950 IF VALID-1ST-FEED-TIME NEXT SENTENCE ELSE MOVE 'X' TO
04960 E-KEY (14) MOVE ALL ' ' TO FIT.
04970 *
04980 * FEED1-TYPE-CK VALIDATES CC 35-36.
04990 *
05000 FEED1-TYPE-CK.
05010 IF VALID-FEED1-TYPE NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (15)
05020 MOVE ALL ' ' TO F11.
05030 *
05040 * FEED2-B-CK VALIDATES CC 37-38.
05050 *

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05060 FEED2-B-CK.
05070 IF MO-2ND-FEED-DAY AND MO-2ND-FEED-TIME GO TO FEED3-B-CK.
05080 IF VALID-2ND-FEED-TIME NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (17)
05090 MOVE ALL ' ' TO F20 GO TO FEED2-T-CK.
05100 *
05110 * FEED2-T-CK VALIDATES CC 39-42 AS WELL AS ITS RELATIONSHIP TO CC 29-34.
05120 *
05130 FEED2-T-CK.
05140 IF AK-COLONY AND AK-2ND-FEED-TIME GO TO FEED2-TYPE-CK.
05150 IF VALID-2ND-FEED-TIME NEXT SENTENCE ELSE MOVE 'X' TO E-KEY
05160 (19) MOVE ALL ' ' TO F21.
05170 IF FEED1-DAY = FEED2-DAY AND (FEED2-TIME NOT > FEED1-TIME)
05180 MOVE 'X' TO E-KEY (18) MOVE ALL ' ' TO F21.
05190 *
05200 * FEED2-TYPE-CK VALIDATES CC 43-44 AS WELL AS ITS RELATIONSHIP TO
05210 * CC 35-36.
05220 *
05230 FEED2-TYPE-CK.
05240 IF VALID-FEED2-TYPE NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (20)
05250 MOVE ALL ' ' TO F22 GO TO FEED3-B-CK.
05260 IF TYPE-FEED01 = TYPE-FEED02 MOVE 'X' TO E-KEY (16) MOVE ALL
05270 ' ' TO F21 F22.
05280 *
05290 * FEED3-B-CK VALIDATES CC 45-46.
05300 *
05310 FEED3-B-CK.
05320 IF MO-3RD-FEED-DAY AND MO-3RD-FEED-TIME GO TO ROOM-CK.
05330 IF VALID-FEED3-DAY NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (18)
05340 MOVE ALL ' ' TO F30 GO TO FEED3-T-CK.
05350 *
05360 * FEED3-T-CK VALIDATES CC 47-50 AS WELL AS ITS RELATIONSHIP TO
05370 * CC 37-42.
05380 *
05390 FEED3-T-CK.
05400 IF AK-COLONY AND AK-3RD-FEED-TIME GO TO FEED3-TYPE-CK.
05410 IF VALID-3RD-FEED-TIME NEXT SENTENCE ELSE MOVE 'X' TO
05420 E-KEY (22) MOVE ALL ' ' TO F31.
05430 IF FEED2-DAY = FEED3-DAY AND (FEED3-TIME NOT > FEED2-TIME)
05440 MOVE 'X' TO E-KEY (21) MOVE ALL ' ' TO F31.
05450 *
05460 * FEED3-TYPE-CK VALIDATES CC 51-52 AS WELL AS ITS RELATIONSHIP TO
05470 * CC 43-44.
05480 *
05490 FEED3-TYPE-CK.
05500 IF VALID-FEED3-TYPE NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (23)
05510 MOVE ALL ' ' TO F32 GO TO ROOM-CK.
05520 IF TYPE-FEED02 = TYPE-FEED03 MOVE 'X' TO E-KEY (17) MOVE ALL
05530 ' ' TO F22 F31.
05540 *
05550 * ROOM-CK VALIDATES CC 53-54.
05560 *
05570 ROOM-CK.
05580 IF VALID-ROOM-NO NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (24)
05590 MOVE ALL ' ' TO RM.
05600 *
05610 * L-M-T-CK VALIDATES CC 55-57.
05620 *
05630 L-M-T-CK.
05640 IF VALID-LARVAL-HIGH NEXT SENTENCE ELSE MOVE 'X' TO E-KEY
05650 (25) MOVE ALL ' ' TO LKT.
05660 *
05670 * L-L-T-CK VALIDATES CC 58-60 AS WELL AS ITS RELATIONSHIP TO CC 55-57.
05680 *
05690 L-L-T-CK.
05700 IF VALID-LARVAL-LOW NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (26)
05710 MOVE ALL ' ' TO LLT.
05720 IF E-KEY (25) = 'X' OR E-KEY (26) = 'X' GO TO A-M-T-CK.
05730 IF LARVAL-HIGH-TEMP < LARVAL-LOW-TEMP MOVE 'X' TO E-KEY (27)
05740 MOVE ALL ' ' TO LMT LLT.
05750 *
05760 * A-M-T-CK VALIDATES CC 61-62.
05770 *
05780 A-M-T-CK.
05790 IF 000B-HIGH-TEMP NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (28)
05800 MOVE ALL ' ' TO AHT.
05810 *
05820 * A-L-T-CK VALIDATES CC 63-64 AS WELL AS ITS RELATIONSHIP TO CC 61-62.
05830 *
05840 A-L-T-CK.
05850 IF 000B-LOW-TEMP NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (29)
05860 MOVE ALL ' ' TO ALT.
05870 IF E-KEY (28) = 'X' OR E-KEY (29) = 'X' GO TO A-M-H-CK.
05880 IF ADULT-HIGH-TEMP < ADULT-LOW-TEMP MOVE 'X' TO E-KEY (30)
05890 MOVE ALL ' ' TO AHT ALT.
05900 *
05910 * A-M-H-CK VALIDATES CC 65-66.
05920 *
05930 A-M-H-CK.
05940 IF VALID-HIGH-RH NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (31)
05950 MOVE ALL ' ' TO AHR.
05960 *
05970 * A-L-H-CK VALIDATES CC 67-68 AS WELL AS ITS RELATIONSHIP TO CC 65-66.
05980 *
05990 A-L-H-CK.
06000 IF VALID-LOW-RH NEXT SENTENCE ELSE MOVE 'X' TO E-KEY (32)
06010 MOVE ALL ' ' TO ALR.
06020 IF E-KEY (31) = 'X' OR E-KEY (32) = 'X' GO TO METH-CK.
06030 IF ADULT-HIGH-RH < ADULT-LOW-RH MOVE 'X' TO E-KEY (33) MOVE
06040 ALL ' ' TO AHR ALR.
06050 *
06060 * METH-CK VALIDATES CC 69-73.
06070 *

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06080 METH-CK.
06090 IF BOTH-ZEROS GO TO DISP1-CK.
06100 IF VALID-C-METHOD NEXT SENTENCE ELSE MOVE 'X' TO E-KET (34)
06110 MOVE ALL ' ' TO CM.
06120 IF ACCEPTABLE-RANGE NEXT SENTENCE ELSE MOVE 'X' TO E-KET (35)
06130 MOVE ALL ' ' TO WF.
06140 *
06150 * DISP1-CK VALIDATES CC 74.
06160 *
06170 DISP1-CK.
06180 IF DISP1-VALID NEXT SENTENCE ELSE MOVE 'X' TO E-KET (36) MOVE
06190 ALL ' ' TO D1.
06200 *
06210 * D1-DAT-CK VALIDATES CC 75-76.
06220 *
06230 D1-DAT-CK.
06240 IF VALID-DISP1 NEXT SENTENCE ELSE MOVE 'X' TO E-KET (37) MOVE
06250 ALL ' ' TO D10.
06260 *
06270 * DISP2-CK VALIDATES CC 77.
06280 *
06290 DISP2-CK.
06300 IF DISP2-VALID NEXT SENTENCE ELSE MOVE 'X' TO E-KET (38) MOVE
06310 ALL ' ' TO D2.
06320 *
06330 * D2-DAT-CK VALIDATES CC 78-79.
06340 *
06350 D2-DAT-CK.
06360 IF VALID-DISP2 NEXT SENTENCE ELSE MOVE 'X' TO E-KET (39) MOVE
06370 ALL ' ' TO D20.
06380 *
06390 * C-TYPE-CK VALIDATES CC 80.
06400 *
06410 C-TYPE-CK.
06420 IF CARD-TYPE-OK NEXT SENTENCE ELSE MOVE 'X' TO E-KET (41)
06430 MOVE ALL ' ' TO CT.
06440 *
06450 * CROSSB-CK IS THE BEGINNING OF THE MAJOR CROSS-FIELD EDIT VALIDATIONS
06460 * RELATIONSHIPS BETWEEN FIELDS IN THE TRANSACTION RECORDS. THIS
06470 * CHECK RUNS THRU CONTINUE1 AND TERMINATES THE BTANORAB
06480 * TRANSACTION EDITS.
06490 *
06500 CROSSB-CK.
06510 IF (RECORD-TYPE = '1' OR '2') AND (FILE-TYPE = '1' OR '2' OR
06520 'B') NEXT SENTENCE ELSE MOVE 'X' TO E-HEY (43) MOVE ALL ' '
06530 TO FT RT.
06540 IF AK-COLOMT AND (ROOM-MO = '03' OR '09') MOVE 'X' TO
06550 E-KET (44) MOVE ALL ' ' TO SC RM.
06560 IF METHMO-HO-FLIES AND HO-FLIEB GO TO CONTINUE.
06570 IF HO-FLIES AND NOT METHOD-HO-FLIEB MOVE 'X' TO E-KET (45)
06580 MOVE ALL ' ' TO CM WF.
06590 CONTINUE.
06600 IF ADULTS-TO-BOTH1 AND NOT ADULTS-TO-00TH2 MOVE 'X' TO E-KEY
06610 (50) MOVE ALL ' ' TO D1 D2.
06620 IF ADULTS-TO-BOTH2 AND NOT ADULTS-TO-BOTH1 MOVE 'X' TO
06630 E-KEY (50) MOVE ALL ' ' TO D1 D2.
06640 IF BLANK-VALUE AND NOT 000B-W-BLANK MOVE 'X' TO E-HEY (51)
06650 MOVE ALL ' ' TO D1 D2.
06660 IF DISPOSITION1 NEXT SENTENCE ELSE GO TO CONTINUE1.
06670 IF DISPOSITION2 = ' ' OR '1' OR '3' NEXT SENTENCE ELSE MOVE
06680 'X' TO E-KET (40) MOVE ' ' TO D1 D2.
06690 CONTINUE1.
06700 IF ADULTS-TO-BOTH2 AND DISP2-BAY < DISP1-DAT MOVE 'X' TO
06710 E-KEY (52) MOVE ALL ' ' TO B10 D2B.
06720 IF SERIAL-CODE = 'AA' AND BASE-DATE < '000000' MOVE 'X' TO
06730 E-KEY (53) MOVE ALL ' ' TO SC B1 B0.
06740 IF SERIAL-CODE = 'AK' AND BASE-DATE < '73009' MOVE 'X' TO
06750 E-KET (53) MOVE ALL ' ' TO SC B1 B0.
06760 IF SERIAL-CODE = 'AM' AND BASE-DATE < '74275' MOVE 'X' TO
06770 E-KET (53) MOVE ALL ' ' TO SC B1 B0.
06780 IF SERIAL-CODE = 'AB' AND BASE-DATE < '73157' MOVE 'X' TO
06790 E-KEY (53) MOVE ALL ' ' TO SC B1 B0.
06800 IF SERIAL-CODE = 'AC' AND BASE-DATE < '7326B' MOVE 'X' TO
06810 E-KET (53) MOVE ALL ' ' TO SC B1 B0.
06820 IF SERIAL-CODE = 'AD' AND BASE-DATE < '73280' MOVE 'X' TO
06830 E-KEY (53) MOVE ALL ' ' TO SC B1 B0.
06840 IF SERIAL-CODE = 'AE' AND BASE-DATE < '7315B' MOVE 'X' TO
06850 E-KET (53) MOVE ALL ' ' TO SC B1 B0.
06860 IF SERIAL-CODE = 'AF' AND BASE-DATE < '72010' MOVE 'X' TO
06870 E-KEY (53) MOVE ALL ' ' TO SC B1 B0.
06880 IF SERIAL-CODE = 'AO' AND BASE-DATE < '72009' MOVE 'X' TO
06890 E-KEY (53) MOVE ALL ' ' TO SC B1 B0.
06900 IF SERIAL-CODE = 'AI' AND BASE-DATE < '74275' MOVE 'X' TO
06910 E-KEY (53) MOVE ALL ' ' TO SC B1 B0.
06920 IF SERIAL-CODE = 'AJ' AND BASE-DATE < '74214' MOVE 'X' TO
06930 E-KET (53) MOVE ALL ' ' TO SC B1 B0.
06940 IF SERIAL-CODE = 'AL' AND BASE-DATE < '75142' MOVE 'X' TO
06950 E-KEY (53) MOVE ALL ' ' TO SC B1 B0.
06960 IF SERIAL-CODE = 'AM' AND BASE-DATE < '75142' MOVE 'X' TO
06970 E-KET (53) MOVE ALL ' ' TO SC B1 B0.
06980 IF SERIAL-CODE = 'AN' AND BASE-DATE < '75142' MOVE 'X' TO
06990 E-HEY (53) MOVE ALL ' ' TO SC B1 B0.
07000 IF SERIAL-CODE = 'AO' AND BASE-DATE < '75142' MOVE 'X' TO
07010 E-KET (53) MOVE ALL ' ' TO SC B1 B0.
07020 IF SERIAL-CODE = 'AP' AND BASE-DATE < '75256' MOVE 'X' TO
07030 E-KEY (53) MOVE ALL ' ' TO SC B1 B0.
07040 *
07050 * REC-PRT. IN THIS PARAGRAPH, RECORDS WITH ERRORS ARE IDENTIFIED
07060 * AND PRINTED ALONG WITH U-LINE WHICH UNDERLINES THE BAD FIELDS.
07070 *
07080 REC-PRT.
07090 IF LINEZ > 50 PERFORM HDR-PRT.
07100 IF ERR-KET = ALL '0' GO TO ALMOST-DOME.
07110 MOVE WORK-REC TO BODY WRITE PRT AFTER 2 ADD 3 TO LINE2.
07120 MOVE ' ' TO KD-SW.
07130 ADD 1 TO MROTE.
07140 MOVE U-LINE TO BODY WRITE PRT AFTER HOME.
07150 MOVE SPACES TO BODY WRITE PRT AFTER 1.
07160 ADD 1 TO MERR.
07170 IF MERR > 5689 DISPLAY 'TOO MANY ERRORS, ABORTING' MOVE CLW07208
07180 'X' TO E00.
07190 *
07200 * ERROR-LOOP CONTROLS THE PERFORMANCE OF THE ERROR SEARCH AND
07210 * PRINT PROCEDURE.
07220 *
07230 ERROR-LOOP.
07240 PERFORM KEY-CHECK VARTIMS B FROM 1 BY 1 UNTIL B > 54.
07250 MOVE ALL ZEROS TO ERR-KET.
07260 MOVE ' ' TO KD-SW.
07270 *
07280 * KEY-CHECK CHECKS EACH OCCURRENCE OF E-KEY (XX) LOOKING FOR A VALUE
07290 * 'X' AND PRINTING ERROR MESSAGE (XX) WHEN ENCOUNTERED.
07300 *
07310 KEY-CHECK.
07320 IF LINEZ > 50 PERFORM KDR-PRT MOVE 'X' TO KD-SW.
07330 IF KD-SW = 'X' MOVE SPACES TO 000Y WRITE PRT AFTER 1 ADD 1
07340 TO LINEZ MOVE ' ' TO KD-SW.
07350 IF E-KET (B) = 'X' MOVE E-M (B) TO BODY WRITE PRT AFTER 1
07360 ADD 1 TO LINEZ.
07370 MOVE SPACES TO BODY.
07380 *
07390 * ALMOST-DOME. HERE RECORDS THAT ARE FOUND TO BE ERROR FREE ARE
07400 * WRITTEN TO THE MASTER FILE.
07410 *
07420 ALMOST-DOME.
07430 MOVE ZEROS TO J-DAT-BASE ERROR-KET.
07440 MOVE SPACES TO WORK-REC U-LINE.
07450 GO TO MAIN-XIT.
07460 *
07470 * 22-CHECK. IN THE CASE OF 22 COLOMT DATA, ONLY PART OF THE EDITS
07480 * ARE PERFORMED. THOSE 22 RECORDS ARE PROCESSED THRU 22-EXIT AND
07490 * THEN PASSED TO THE ERROR PRINT PORTION OF MAIN.
07500 *
07510 22-CHECK.
07520 IF DAT-366 AND LEAP-TEAR GO TO 22-A.
07530 IF YEAR-VALID NEXT SENTENCE ELSE MOVE 'X' TO E-KET (07) MOVE
07540 ALL ' ' TO B1.
07550 IF DAT-VALID NEXT SENTENCE ELSE MOVE 'X' TO E-KET (08) MOVE
07560 ALL ' ' TO 0D.
07570 22-A.
07580 IF VALID-ROOM-MO NEXT SENTENCE ELSE MOVE 'X' TO E-HEY (24)
07590 MOVE ALL ' ' TO RM.
07600 IF VALID-LARVAL-HIGH NEXT SENTENCE ELSE MOVE 'X' TO E-KEY
07610 (25) MOVE ALL ' ' TO LMT.
07620 IF VALID-LARVAL-LOW NEXT SENTENCE ELSE MOVE 'X' TO E-KET (26)
07630 MOVE ALL ' ' TO LLT.
07640 IF E-HEY (25) = 'X' OR E-KET (26) = 'X' GO TO 22-D.
07650 IF LARVAL-HIGH-TEMP < LARVAL-LOW-TEMP MOVE 'X' TO E-KEY (27)
07660 MOVE ALL ' ' TO LMT LLT.
07670 22-B.
07680 PERFORM A-H-T-CH.
07690 IF 000B-LOW-TEMP NEXT SENTENCE ELSE MOVE 'X' TO E-KET (29)
07700 MOVE ALL ' ' TO ALT.
07710 IF E-KET (28) = 'X' OR E-KEY (29) = 'X' GO TO 22-C.
07720 IF ADULT-HIGH-TEMP < ADULT-LOW-TEMP MOVE 'X' TO E-KET (30)
07730 MOVE ALL ' ' TO AHT ALT.
07740 22-C.
07750 PERFORM A-K-K-CH.
07760 IF VALID-LOW-RH NEXT SENTENCE ELSE MOVE 'X' TO E-KET (32)
07770 MOVE ALL ' ' TO ALR.
07780 IF E-KEY (31) = 'X' OR E-KET (32) = 'X' GO TO 22-EXIT.
07790 IF ADULT-HIGH-RK < ADULT-LOW-RH MOVE 'X' TO E-KET (33) MOVE
07800 ALL ' ' TO AKR ALR.
07810 22-EXIT.
07820 EXIT.
07830 MAIN-XIT.
07840 EXIT.
07850 *
07860 * HOR-PRT IS THE PERFORMED HEADER PRINT ROUTINE.
07870 *
07880 HDR-PRT.
07890 MOVE HEAD1 TO BODY WRITE PRT AFTER TOPLINE.
07900 MOVE HEAD2 TO BODY WRITE PRT AFTER 1.
07910 MOVE HEAD3 TO BODY WRITE PRT AFTER 1.
07920 MOVE HEAD4 TO BODY WRITE PRT AFTER 1.
07930 MOVE HEAD5 TO BODY WRITE PRT AFTER 2.
07940 MOVE HEAD6 TO BODY WRITE PRT AFTER 1.
07950 MOVE 07 TO LINEZ.
07960 READT

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Appendix C.—Summary of the Retrieval-Request Document Based on Data Entered in the Record Document (Fig. 3) and Codes in Appendix A

Information requested

Data input required

1. Pupal production (69-73). ⁵	Julian dates (as coded in columns 14-18) for the time interval (time span). Colony code (as coded in columns 3-4). Time unit ⁶ —1=day, 2=week, 3=month, 4=quarter, and 5=year. Plot—1=yes, 2=no.
2. Adult holding temperatures and relative humidities for a specific colony or room (61-68).	Julian dates for the time interval. Colony code and room-number code (as coded in columns 53-54) number code only. Time unit (as above).
3. Larval rearing-temperatures for a specific colony or room (55-60). ⁷	Julian dates for the time interval. Colony code and room-number code or room-number code only.
4. Larval rearing-temperature history for a specific cage of adult insects (55-60). ⁸	Colony code. Large-cage number or large-cage number and subcage number, (as coded in columns 5-8 and 9-11). Number of days required for larval development.
5. Data listing by date for a specific colony (1-80).	Julian dates for the time interval. Colony code.
6. Data listing for a specific time interval for all colonies (1-80).	Julian date for the time interval.
7. Data listing for a group of large cages for a specific colony (1-80).	Ranges of large-cage numbers. Colony code.
8. Use of flies (74-79). ⁹	Julian dates for the time interval. Colony code. Time unit (as above).
9. Listing of cages of flies outside a specific temperature range (5-8, 9-11).	Julian dates for the time interval. Colony code. Temperature range (as coded in columns 55-64)—1=larvae (a=high, b=low), 2=adult (a=high, b=low). Number of days required for larval development.
10. Listing of cages for a specific colony that received a specific number of feedings for a specific time interval (5-8, 9-11). ¹⁰	Julian dates for the time interval. Colony code. Number of feedings—1=1, 2=2, 3=3.
11. Universal retrieval program (any of the data in 1-80 by request).	Objective—provide retrieval of data with the fewest restrictions possible imposed by the program, but allow users to restrict the data listed as much as they wish by specifying codes, for particular fields, that must be present for data to be listed. Input—user provides codes or variables acceptable by the current editing program for any of the fields of the colony-production record (fig. 3). The inputs of the computer program correspond to the fields shown in figure 3. As the user

⁵See figure 5 for sample output.

⁶Time unit must be less than or equal to time interval.

⁷See figure 6(A) for sample output.

⁸See figure 6(B) for sample output.

⁹See figure 7(A and B) for sample output.

¹⁰See figure 8(A) for sample output.

Information requested

12. Quality control of size for a specific colony; to list the Julian date of the quality-control sample, the number of pupae per milliliter, mean wing length (mm) \pm S.E. (standard error), and mean dry weight (μ g) \pm S.E.¹¹

Data input required

enters codes in more fields, the listing becomes more restricted (for example, if AA were entered in columns 3 and 4—colony—then the data listing would be only for that colony but would include all data associated with the AA code; if AA were entered in columns 3 and 4, and if the numbers 3398–4240 were entered in columns 5–8—large-cage numbers—then the listing would include all data associated with the AA colony for large cages numbered from 3,398 to 4,240). Also, the computer program accepts inputs of two or more codes or variables or a range of codes or variables for the same field. In such cases, the data will be listed for all codes or variables and/or their ranges, that have been entered as inputs.

- Julian dates for the time interval.
Colony code.
Time unit (as above).
Listing by Julian date—1=yes, 2=no.
Listing by large-cage number—1=yes, 2=no. If yes, enter large-cage number and/or range of numbers.
Plot and printout of primary and secondary polynomial regression equation of dry weight (X-axis) times wing length (Y-axis)—1=yes, 2=no.
Plot and printout of primary and secondary polynomial regression equation of dry weight (X-axis) times the number of pupae per milliliter (Y-axis)—*Culicoides* only; 1=yes, 2=no.

¹¹See figure 8(B) for sample output.

Appendix D.—Computer Programs Used to Operate the Information Retrieval System

Program	Contents or programs
CLIST	
S FORMAT	Creates SAS-format module used by statistical analysis programs.
STAT	Executes statistical-analysis programs.
STATBIG	Executes large statistical-analysis programs.
CNTL	
DISKTape	Copies colony-production data base from disk to tape.
TAPEDISK	Copies colony-production data base from tape to disk.
LOADSTAT	Copies statistical programs, format module, and CLIST programs from tape to disk.
EXEC.COLVERIF	Executes colony-production editing program and error-message file from disk.
LOADEXEC.COLVERIF ..	Executes colony-production editing program and error-message file from tape.
LOAD MODULES	
COLPROD	Contains editing program COLVERIF.
FORMAT	Contains SAS-format program S FORMAT.
SOURCE PROGRAMS	
COLVERIF	Colony production editing program.
S FORMAT	Format program for SAS statistical-analysis programs.
STATPGMS.A	Statistical-analysis programs (see appendix C, 1-4 and 12).
STATPGMS.B	Statistical-analysis programs (see appendix C, 6-12).
DATA FILES	
ERRMSGs	File of the texts of error messages printed out by colony-production editing program.
NEW.COLONY	Colony-production data for 1978.
TAD17	Colony-production data for 1974-77.

Systems Management of Insect-Population-Suppression Programs Based on Mass-Production of Biological-Control Organisms

By N. C. Leppla¹

Introduction

Effective management defines and organizes the variables that comprise a functional system and uses the system to maintain itself. Having discovered that a finely tuned organization will almost run itself, successful managers have a nearly intuitive sense of order. So, rather than managing hectically and anxiously from crisis to crisis, they have become professionals who anticipate and almost relish most administrative challenges.

One such challenge is to develop new technologies that can be integrated with existing ones for reducing the impact of agricultural pests. The result is IPM (integrated pest management), which includes chemical control, plant and animal resistance, cultural practices, and biological control (parasites, predators, pathogens, sterile-insect technique, and genetic manipulation). Most of these techniques depend on having large numbers of laboratory-reared insects for their development and implementation. It would be wonderful if we could simply collect some insects, rear and sterilize them, spray them back into the field, and watch the pest population disappear. Unfortunately, there are no insecticidelike "magic bullets." No single set of rules and procedures for managing population-suppression programs based on mass-produced organisms exists; nor should it, since the contributing elements can be fitted together in various ways to solve different problems. This paper describes elements of a generalized system for rearing and utilizing insects that can be used as a framework for various programs with various needs and purposes.

System Components

This conference has documented recent advances and existing capabilities for handling the primary elements of insect-rearing and utilization programs. It has also emphasized the importance of organizing these elements into functional systems that can be managed effectively.

This approach of generating and operating unified systems rather than dividing them into smaller, ostensibly more manageable parts is a major technological revolution (Kavanau et al. 1971). Such a system is more than an aggregation of interrelated elements; it has a life of its own so that it reacts as a unit to adjustments in its parts. The system's hierarchal structure can provide several operational levels. Also, because the system requires cooperative effort, it may become the means for accommodating divergent attitudes, conflicting motives, and dissimilar ideologies.

The first step in developing an integral system is to arrange the existing subsystems into a functional pattern. For an insect-population-suppression program, the primary functions are operation, quality control, and perpetuation. "Operation" includes colonization, production, and utilization, the subsystems that directly contribute to organizational output. "Colonization" is primarily strain development; production and utilization emphasize process engineering. "Quality control" is the monitoring and evaluation of insectary products and their use, and it affects all phases of the program. "Perpetuation" includes all the elements of effective management that facilitate interactions among the subsystems. These subsystems and their elements are characterized by relative permanence; but the operations, procedures, and associated facilities and equipment vary as the system evolves.

Operation

Colonization.—The first element of the colonization subsystem is characterization of the target population. The pest's geographical and temporal distributions, host preferences, life history, etc., are determined by conducting ecological surveys. The basis for a strategy for behavioral or genetic control is derived from these data. If, for example, the object is to disrupt reproduction, all pertinent prereproductive and postreproductive processes must be understood. Once the pest has been characterized, the laboratory colony can be established with insect collections of the appropriate composition. Desired genetic traits are preserved by relaxing environmental stress within the insectary and by adopting a suitable maintenance strategy such as perpetual isolation, hybridization, periodic infusion, or replacement. The

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characterized insect becomes the biological standard for measuring the effects of production and utilization operations.

Production.—The elements of production are rearing, engineering, and management. “Rearing” includes acquisition and storage of materials; diet formulation and preparation; egg collection, treatment, and placement in containers; larval development; removal and distribution of pupae; adult maintenance; and microbial control. “Engineering” encompasses facilities, equipment, instrumentation (monitoring and control of processes and environments), and maintenance. “Management” optimizes, organizes, and allocates all available resources to sustain the colony.

Utilization.—“Utilization” is the treatment, transport, and deployment of population-suppression organisms. Treatment may involve sterilization by irradiation, chemicals, genetic alteration, or physical stress; also, it usually includes some kind of marking (dye, isotopes, etc.) and preconditioning (chilling, photoperiodic entrainment, acclimation, etc.). Obviously, the insects must be transported quickly and under the best physiological conditions, an operation that is technically simple but often difficult to accomplish in practice. “Deployment” is the development and application of procedures and equipment for insect release. Like production, utilization requires an integration of scientific and engineering expertise (for example, see Smith 1977).

Quality control

“Quality control” is the monitoring of colonization, production, and utilization operations and their effects on the products. Monitoring techniques are developed and implemented, the data are periodically evaluated, and recommendations are made for improving the system. But, once tests and standards have been established, routine measurements are made as procedures directly involved with performing an operation. So quality control works like a servomechanism. A solid-state temperature controller, for example, monitors the ambient air with sensors, evaluates changes in electrical current within its circuits, and actuates devices to heat or cool the air until conditions stabilize. Quality control operates similarly to maintain desired conditions; but, since it provides tolerances and standards, it is more flexible than a single regulatory device. It actually coordinates the operations and insures the maintenance of acceptable standards of colonization, production, and utilization.

Perpetuation

The ultimate goal of program management is to establish a dynamic system that eliminates any long-term

dependence on individual managers and builds the organization around program goals. An insect-population-suppression program, like any business corporation, should be created to preserve the organization's continuity and extend its capabilities beyond human limits. Management must therefore think in terms of what operations are needed and what procedures must be followed rather than available skills and training. Certainly, key roles will emerge and be assumed by responsible people, but performance and productivity should never be limited by the limitations of the personnel.

If we assume that those associated most closely with an operation know most about it, then, clearly, subsystems of an insect-population-suppression program should be managed internally. But, participatory management is needed for the refinement and perpetuation of the subsystems (King 1975). People who concentrate on implementing one set of operations may not be aware of technologies that could improve their effectiveness and are usually unable to see their relative importance. So it is advantageous to cultivate an interdependent partnership and integrate available expertise to solve mutual problems. Management provides this capability to achieve this integration through organization, support, communication, and regulation.

Invariably, successful managers are supportive. They not only allocate resources where needed, but they also attempt to eliminate obstacles to employee success such as poor work environments (ones that are hazardous, inflexible, or constantly interrupted), deficiencies in training or experience, or inadequate coordination. Most employees will achieve their potential if such obstacles are moderated or removed and if they are given a little motivation in the form of material and emotional satisfaction.

Management is sometimes characterized as a sinister activity, wholly preoccupied with the formulation and enforcement of regulations. Indeed, some antiquated managers still consider this to be their primary function. But enlightened managers know that the object of an organized effort is productivity not regulation. They concentrate on defining and measuring productivity and regulating only those activities needed to achieve a suitable quantity and quality of work. In the case of pupal irradiation, for example, management is concerned with the dosage and number of sterilized pupae and not with maintenance schedules, time cards, dress codes, etc., of the employees. Guidelines should specify output and only those personal habits that directly affect it. This approach maximizes the flexibility necessary for handling contingencies and preserving employee autonomy, dignity, and professionalism.

Effective communication is the key to accomplishing

organizational, supportive, and regulatory goals because it links subsystem elements, provides a way to incorporate new technology, and facilitates the enforcement of policies. It is the means by which deficiencies are corrected and achievements recognized. For communication to be effective, people who work together should have frequent informal meetings; staff meetings should be held less often but according to an appropriate fixed schedule. Employees should always understand their roles in the organization and never doubt the importance of their contributions.

Factors Complicating Systems Management

Population-suppression programs that depend on the efficient production and utilization of biological-control organisms often appear unmanageable because they are inherently complex, relying on technology that is derived from many interrelated disciplines. Since coordinating these disciplines is difficult, the field has remained unnecessarily compartmentalized. For example, it is impossible to deal with diets and containerization without considering microbial contamination, and biological and engineering problems are inseparable when rearing, transport, and release are automated. So management and quality control are necessary to every aspect of a program.

Another problem that hinders progress and demands management coordination is the divergence of specialized fields. Scientists, engineers, administrators, educators, merchants, and other specialists may agree that the purpose of insect rearing is to provide a dependable supply of organisms that meet acceptable biological standards, but they have somewhat different attitudes and priorities concerning the allocation of resources. The entomologist, for example, is inclined to accept the dynamic and empirical processes that dictate relatively frequent changes in materials and equipment, but the administrator may question the expense, and the engineer might actually propose that the insects be changed to save the hardware! These specialists are not inflexible, but they function in different contexts whose compatibility they do not always recognize. So they must be linked by effective management. (For a worthwhile discussion of the role of engineering in developing new technology, see Bailey 1978.)

Other problems management will have to deal with include agricultural practices incompatible with biological-control strategies, national and international politics, long-term financial support, and imbalances in the proportions of basic research and dependent technology. These considerations and others have somehow dictated that massive programs be controlled by a few managers and thus become limited in scope by their visions and talents. But the most appropriate way to plan, operate, analyze, and perpetuate these complex programs is to adapt the concepts and principles of systems management. Systems management concentrates critical evaluation on the program rather than on its individual elements. The details can be organized and controlled by means of modern computer technology, and the system can serve as its own model for testing creative ideas without disrupting existing operations and making costly mistakes. Also, cooperation and interdependence are promoted through effective coordination. Management must unify the complex contributing elements and provide insect-population-suppression programs that will meet agriculture's future challenges. (For a humorous but pointed discussion of management, see Ettinger 1970.)

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The Insectary Manager

By W. R. Fisher¹

Introduction

Rearing animals in the laboratory presents challenges and problems unlike those associated with the production of inanimate objects. This is especially true with the production of insects. First, each species goes through distinct developmental stages that have different life styles and different requirements for temperature, relative humidity, lighting, diet, population density, containment, and sanitation. So the environment in the rearing facility must cater to the needs of each stage, particularly immatures, whose needs are often radically different from those of the adult. Second, insects in culture must be protected against pathogens, parasites, and predators, which can affect quality, reduce yields, and disrupt scheduling. And a cannibalistic species must be protected from itself. Third, during the course of production, insects must be handled when, for example, they are placed on diet, moved to a different container, or surface-sterilized. This handling must be done carefully because eggs and pupae are easily smashed, and soft-bodied larvae can be easily punctured. Fourth, insect populations are characterized by an element of inherent variability that can manifest itself, regardless of environmental constancy, as subtle differences in morphology, developmental rates, and behavior. The type and degree of variation depend on factors such as the stage of colonization and the time of year. So identification of significant inconsistencies in insect quality becomes difficult. Also, variability is increased by many elements in the rearing program itself, including handling of insects and the types of diet. Correlating anomalies with specific causes and correcting for them is therefore a complex task. So, the challenges and problems associated with insect-production programs provide a unique management opportunity that requires trained, experienced individuals to establish and maintain a quality production program.

Training and Experience

Two factors necessitate a higher level of training for future insectary managers: the need for more efficient production of quality insects and the increasing recognition that manipulation of variables in the rearing pro-

gram will be needed to enable adjustment of that quality to satisfy the specific needs of each release program. The insectary manager should have academic training in disciplines that are basic to the major areas of insect production, such as the insects, their environment, and insectary personnel. Courses in general entomology, insect physiology, pathology, and behavior provide an understanding of insect life cycles, development, morphology, and diagnosis and treatment of disease, and they form the basis for evaluating production efficiency and insect quality. Courses dealing with insect ecology, general microbiology, and general nutrition will illustrate the effects of variables such as temperature, relative humidity, and population density on insect growth and development. They will also demonstrate microbiological concepts, sanitary techniques, and nutritional requirements of living organisms. A course covering maintenance of environmental conditions will provide an understanding of heating, cooling, and humidification systems and how they are controlled and monitored. Lastly, a basic course in personnel management will help the manager in scheduling activities and organizing available labor.

Formal coursework in understanding basic concepts is valuable, but practical experience and training remain the primary means of developing expertise in insect rearing. Firsthand observations allow the manager to get a feel for the program. By handling insects, performing procedures, and identifying problem areas, the manager is better able to evaluate the performance of the facility, the equipment, and the personnel. For example, one can read the recipe of ingredients and amounts used to prepare an artificial diet, but one must observe the actual blending action, texture, and gelling characteristics of the mixture to be able to decide how to insure consistent batches. Likewise, the manager must participate in the program directly for a considerable length of time to gain the knowledge needed for making decisions about subjective matters such as the cause of abnormal fluctuations in yield or insect quality or the course of action to take in response to an outbreak of disease or dietary contamination. With this experience, the manager should develop an intuitive sense about the operations necessary for the most appropriate response at the most advantageous time. An inexperienced manager might overreact to an apparent problem, making unnecessary or premature adjustments in the program.

Responsibilities

The insectary manager must be an active participant in the rearing program. He must know production require-

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ments, as well as the responsibilities and activities of all employees. He must know how to perform procedures and how to use equipment. The manager must also insure that all items needed for successful and safe completion of all tasks are available. These include lab coats, gloves, data books, stationary supplies, etc. Ordering any supplies, especially dietary ingredients or containers, should be done by the manager well in advance of their use to avoid disruption of the program because of unexpected delays in availability or shipping.

Often, the items needed for successful rearing are unavailable from suppliers and must be custom-built to specifications based on the biology of the insect and other considerations. For instance, one manager recognized that a cannibalistic species needed to be reared in individual containers. At the same time, she realized that this procedure depended on expensive, nonreusable plastic cups and a labor-intensive effort to pour diet, plant eggs, cap cups, and harvest pupae. She investigated a more efficient, reusable, multicellular system that would be a significant savings in labor and money. Reports indicated, however, that these units were made of brittle polystyrene plastic with square-cornered cells that prohibited adequate cleaning before reuse. The cell units were heat labile and could not be sterilized in a steam autoclave. Disinfection in bleach solution did not adequately destroy contaminants. This inadequate disinfection was of major concern to the manager because she wanted to reduce the incidence of disease organisms and dietary contamination. She concluded that a new material was needed that would be inexpensive and able to withstand sterilization in an autoclave. Individual cells should be round and seamless to aid cleaning and should be large enough to contain enough diet for development and pupation but small enough for efficient production. Material covering the top of the cell unit should allow adequate gas exchange for maintaining the necessary microclimate in cells, and, at the same time, it must be of small pore size to keep neonates from escaping. It must also be rugged enough to withstand the damage from mandibles of late-instar larvae. The final product consisted of liquid resin poured into a reusable, flexible mold that was peeled away after the resin had hardened. The covering material was porous polypropylene, commonly used for containing insects. Both these materials are autoclavable. This example illustrates the many variables that must often be considered by the manager when making decisions about the rearing program.

Beyond the relatively obvious duties, one of the manager's primary responsibilities is to be aware of existing or potential problems such as contamination or equipment failure. He may then solve such problems or reduce their impact should they threaten the program. First, though, he must enforce regulations designed to

reduce the occurrence of problems such as the spread of disease throughout the facility. For example, unnecessary personnel must be restricted from sanitary areas, and the restriction must be enforced without exception. Even the best rearing program cannot compensate for valid rules that are not enforced. This example is an illustration of the kind of procedures the manager should follow to solve problems by treating their causes and not merely by treating their symptoms. It is, in this illustration, more advantageous to prevent disease by restricting the movement of personnel than it is to treat disease symptoms by adding to the diet antimicrobial chemicals that may affect insect quality.

Problems may develop as a result of equipment failure. Knowing what to do in such an emergency is often difficult unless prior thought has been given to potential causes and alternative courses of action. Figure 1 is an example of such a plan for dealing with potential problems. In this dichotomously-branching flow chart, a problem with the air-conditioning system causes an alarm to be sounded. The manager follows the chart step-by-step

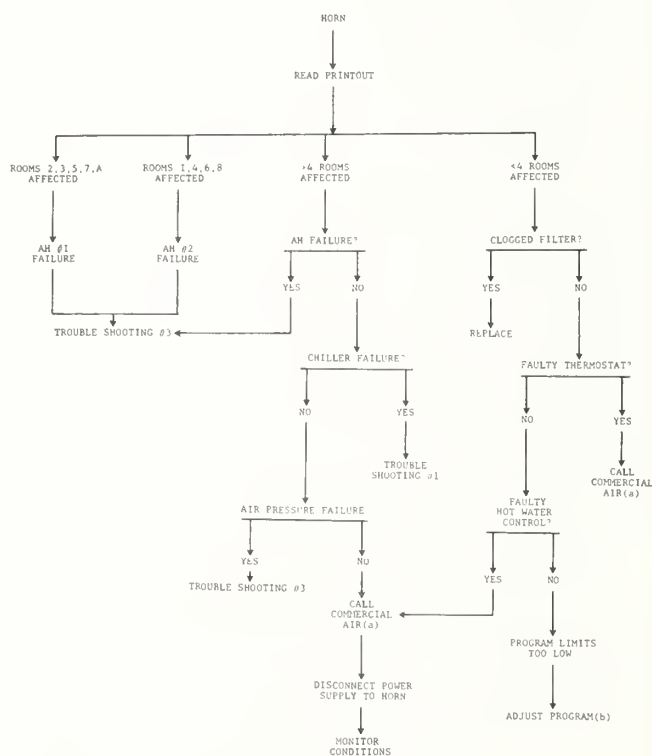


Figure 1.—“Trouble Shooting Chart #2: Data Logger Alarm System for Abnormal Temperatures in the Insectary” (from Wiegand 1978). A person using this chart should be able to identify the cause of problems associated with the air-conditioning system.

to determine what action to take to resolve the problem. Familiarity with the system, knowledge of the location of the chiller, air handlers, boiler, etc., and insuring that extra supplies such as fan belts and prefilters are on hand are the responsibility of the manager. If the problem cannot be resolved easily, the chart indicates what professionals to call. Anticipating potential problems in this manner functions in several important ways: chart development requires that the manager understand the system; the chart itself points very quickly to possible solutions when time is crucial; and it is a written set of guidelines that can be used by other individuals in the absence of the manager.

The insectary manager should anticipate problems and project a set of possible solutions before the problems occur. This is further exemplified in figure 2, which summarizes the contamination potential in a multispecies rearing facility I designed (Fisher 1978; see also "Production of Insects for Industry. The Dow Chemical Rearing Program," by W. R. Fisher). After completing the initial layout and before construction began, I identified potential sources of contamination, mode of entry into the facility and its rooms, and measures to control them. If any major, uncontrolled sources had been detected, the design and procedures could have been changed before completion of the building to insure a more sanitary operation. This type of table is just as important for programs already in operation. Obviously, such anticipation is impossible for all problems, but even the experience in thinking about the more probable ones will be helpful in developing appropriate responses to those that are unexpected.

The most basic requirement for a manager's success is proper training of employees. The initial training should be in a classroom where discussions emphasize rearing philosophy and concepts, including relevance and objectives of the rearing program; the significance of the employee's participation; insect biology, ecology, and nutrition; the presence and transmission of microbial contamination; program efficiency; and insect quality. Beginning training by directly involving an employee in rearing processes may be overwhelming and result in one who does not thoroughly understand the concepts underlying the program.

Training should then shift from the classroom to production areas, where the manager explains the logic of why an activity is performed as well as how it is conducted. This training will make the employee more conscientious in performance of his duties while enabling him to more readily identify developing problems. For example, explaining that surface sterilization of eggs is required because "that's the way it's always been done" is not adequate. This explanation says nothing of how impor-

tant this procedure is to disease prevention or of how it will affect the eggs if not performed properly. The reason for surface sterilization should be clearly stated: "One way disease can be spread from one insect to another is by contamination of the outside of the egg with virus, bacteria, or other micro-organisms. When the larva chews a hole in the egg during hatch, it may eat some of these microbes and become diseased. Soaking the eggs in a bleach solution for 5 minutes destroys these harmful organisms. Part of the eggshell is dissolved in this procedure, but this doesn't hurt the eggs unless they remain in the sterilant too long. Doing so can kill them. So it is very important that this procedure be followed precisely."

The daily routines that the manager establishes for employees must be flexible enough to cover holidays, sick leaves, vacations, etc. When the routine is temporarily altered, the manager then sets priorities and adjusts schedules accordingly. Organization of responsibilities includes consideration of equipment usage so that one employee is not unnecessarily waiting for another to finish. Employee activities should likewise progress logically during the day. For instance, jobs that require sanitary conditions, like preparation of artificial diets, should be completed before operations are begun that create greater contamination potential, such as harvesting pupae or washing dirty containers.

The insectary manager is responsible for regular collection of data so that abnormal trends in production levels and insect quality can be observed. This requires handling data in a way, such as in process-control charting (see "Putting the Control in Quality Control in Insect Rearing," by D. L. Chambers and T. R. Ashley), that quickly and easily illustrates program status. Then, if an anomaly or harmful trend is observed, the manager takes appropriate action. For example, I once observed that the incidence of wing deformities in adults of a lepidopteran species was increasing over time as were reduced fecundity and premature death. Variables suspected of causing this effect were analyzed. I concluded that a lack of polyunsaturated fatty acids in the artificial diet caused the deformities and other symptoms. These were then eliminated by adding raw linseed oil to the diet. As in this example, subtle changes can occur slowly, often imperceptibly when observed on a daily or weekly basis; but they may become manifest as a major problem when data are analyzed over longer periods.

Finally, the insectary manager is the spokesman for the program and the interface between it and the researchers who actually use the insects (see "Management of Insect Production," by Charles P. Schwalbe and O. T. Forrester). He coordinates the requirements of the rearing program with those of the researchers towards a common

Potential Source	Relative Degree of Risk	Control Measures		
1. Air conditioning supply ducts		Absolute filters eliminate over 99% of all microorganisms greater than 0.3 microns		Training in use of pass-throughs
A. Outside air	Moderate	Magnahelic gages indicate effectiveness of filters		Factory installed contamination barrier around autoclave
B. Air recirculated within building	Significant	Ceiling-mounted germicidal lamps reduce air and surface contamination during non-working hours	6. Leakage of contaminants around doors	Refrigerator-type, magnetic seals
2. Auxiliary air flow from fumehoods	Moderate	Hood design insures auxiliary air will not enter rooms Hood has safety switch that will not allow auxiliary air to flow when exhaust fan is not operating	7. Miscellaneous materials taken into critical areas (dietary ingredients, rearing cups, etc)	Automatic mechanism seals closed door to floor
3. Personnel	Very Significant	Critical rooms are restricted to authorized persons Three-way intercom eliminates unnecessary employee traffic Training of personnel in microbiological concepts and sanitary techniques Static foot mats to collect dirt Protocols (hand washing, clean lab coats) required before entering critical areas	8. Reusable materials (larval containers, adult cages)	Materials taken out of shipping boxes before being moved into areas Wash containers with sodium hypochlorite Strict washing procedures Steam-sterilization with direct passage into clean storage
4. Dust and dirt build-up on floor	Moderate	Coved floors for easy cleanup Regular sweeping, vacuuming, and mopping with germicidal detergent	9. General	Sanitation of most plastics in sodium hypochlorite Storage of clean materials in enclosed racks Use of "autoclave tape" to insure sterilizing conditions are met Cleaning hoods and work surfaces with germicidal detergent after use
5. Leakage of contaminants through pass-throughs and double-door autoclave	Significant	Germicidal lamps in pass-throughs well-sealed pass-through doors		Monitor contamination levels with agar plates Empty trash cans each day Pick up escaped insects immediately Building design to insure complete isolation of activities

Figure 2.—Potential sources of contamination in the Dow Insect Production Facility (from Fisher 1978).

goal. So changes in yields, adjustments in insect quality, etc., desired by researchers should be requested of the manager, who can then modify the program accordingly.

Conclusions

The insectary manager links the unique challenges and problems of insect production with the practical application of rearing concepts. He links the control of contamination and the improvement of insect quality with environmental monitoring and manipulation. He links personnel with performance guidelines and effective use of the facility. Finally, he links old, tested procedures and techniques with new ones that improve program efficiency, safety, and capability to produce insects of better quality. Philosophically, this requires that he view the production program as a dynamic system that evolves as new technology and techniques are developed. The manager, as the interface between these ideas and his program, has a thorough understanding of them both and the best perspective for planning and making changes.

Acknowledgments

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Management of Insect Production

By Charles P. Schwalbe and O. T. Forrester¹

Introduction

"Management" is a generally used and deceptively simple word that implies the accomplishment of an end by judicious use of means. But close examination of situations relying heavily on management reveals that the means critical to accomplishment of the end are usually complex and unwieldy. Many of today's managers are ill-equipped to deal with this complexity, even though several training programs, university curriculums, books, etc., are available that deal with the theory and practical applications of management concepts. Necessity often places people with varying degrees of experience and training in management roles. These newborn managers usually do the best they can; but, for several reasons, some are successful and others are not. Failures are tolerable in systems equipped with fail-safes and backups designed to minimize the effects of poor management, but insect-rearing facilities and laboratories are usually austere operations where fail-safes are uncommon. Here we will examine what production managers in insect-rearing operations can do to prevent system failures and show how the various components of insect production depend on each other.

The Production Manager

Anyone responsible for an insect-rearing program is a manager. The manager's degree of responsibility depends mainly on how big and how complex the production system is; it could be small-scale rearing for laboratory and field research programs, pilot-scale production supporting large experiments or pilot projects, or mass rearing in direct support of operational line programs. But basic managerial principles of planning, organizing, monitoring, and upgrading apply to all cases. If these principles are ignored, the rearing operation will probably fail. One person should be clearly responsible for the functioning of the operation and should have the authority to carry out the duties effectively. This person is a production manager.

The production manager is the link among three major units: technology and production groups within the production program and user groups for whom the insects

are produced. The manager insures that the technology group designs a rearing system that will reliably and efficiently produce what the user needs. The manager must also insure that pertinent technical information is available to the program. Designing a rearing operation without technical input from experts (engineers, physiologists, geneticists, pathologists) would be foolish. Still, by using commonsense, a manager can design and improve many operational details; an effective manager will find the most expedient options. The technology group is also instrumental in troubleshooting operations, resolving technical problems, and conducting research for modifying and improving production sequences. A well-directed technology group not only operates in the present, keeping things working, but also develops basic information on innovative, more efficient production methods.

The production manager will also participate in the planning of user programs, especially when large field tests or operational insect-population-suppression programs rely on receiving large numbers of insects regularly. The reared insects will be a resource that line managers will count on for their programs; they must know what rearing capabilities and limitations will be so that they can plan the program's size and timing. In providing such information, the production manager is able to show how the rearing program might affect the user program.

Directing the technology group and helping plan the user program are important duties for production managers, but their most challenging role is to manage the various resources in a production group in a way that is consistent with available technology and user needs. These guide the methods, level, and duration of the rearing program. The production manager must have an effective relationship with the production group so that the technology is used most effectively and the user receives quality insects on time and at the least possible cost.

Elements of Production Management

Production management requires development of several components (objectives, resources, operating plans, review, and technology development and implementation) that, when compiled and coordinated, constitute a unified production plan. This work plan should be carefully prepared because it enables the rearing program to be conducted in a uniform, systematic, and orderly fashion. Failure to carefully consider any one of these components and its place in the total system leaves the rearing pro-

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gram vulnerable to breakdown. And failure to reassess all elements regularly and to redesign them when necessary may result in an outmoded plan.

Objectives

A production program must have established objectives. A statement of objectives, telling how many insects will be ready in a given time, is a necessary first step toward developing plans for other production-management elements. These statements can also tell program participants what is going on and, so, solve the common problem of employees and cooperators not identifying with the purposes of their activities.

Establishing how many insects will be available, and when, is usually straightforward. The manager organizes an annual production schedule (fig. 1). Such a plan identifies production-delivery responsibilities and is the foundation for all other plans. A schedule of this nature is especially useful when the program provides insects of different stages or species to many users.

Objectives also define other important aspects of the production program, especially ways to improve the efficiency of operations. Improved quality standards, increased yields, development of new techniques, implementation of demonstrated techniques, training, improvement of the physical plant, and staffing are all factors to be considered in plotting a course of action. As with the production schedule, the objectives should be specific, quantified, and organized to show how long they will take to fulfill.

Resources

The management of resources used in the production of insects can be complicated. A well-planned, efficient rearing program requires adequate resources, but gross surpluses are wasteful. Any activity will slow when the supply of an essential resource dwindles, and insect rearing is no different. The production manager must devise specific procedures to facilitate management of time, space, personnel, materials, and funds.

Time.—Time-and-motion information is useful in determining how much time is needed for accomplishing certain activities (diet preparation, implanting, harvesting, cleaning, etc.). The production manager should also know how much time will be needed for future events such as colony scale-up, diapause, insect developmental times, purchasing and delivery of supplies, inventory utilization rates, and hiring procedures. Failure to account for possible lags in procurement of dietary ingredients, for example, could easily allow the inventory to run out.

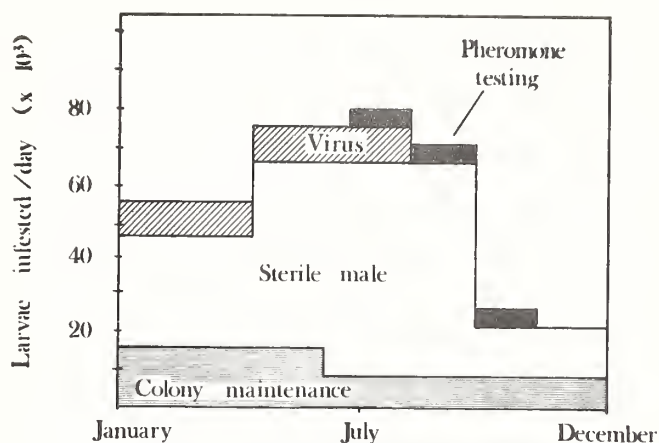


Figure 1.—Stylized annual production schedule.

Space.—Available space must be allocated and managed so that all essential activities (supplies storage, diet preparation, environmentally controlled holding, harvesting, etc.) are accommodated. In arranging space, the production manager must be especially aware of the need for pathogen isolation or containment. Work areas may require special utilities or environmental conditions that will affect space allocation. The manager must also consider what space will be needed for future modifications of existing areas of the facility committed to a certain function. It is not unusual, for instance, for space to be a limiting resource when increased production is needed; all other resources may be available; but, if there is no room to use them in, the amount of insects produced will not increase.

Personnel.—In developing staffing patterns for the program, production managers must plan for the seasonal nature of insect-production operations; so they often rely on temporary employees. Proper training and motivation of these temporary employees, in particular, help to insure that the rearing system continues to work and be efficient. Resourceful managers will make certain that employees know the program's purpose and how they contribute to its accomplishment no matter how insignificant their jobs may seem to be.

Materials.—A wide variety of materials and equipment is used in insect production, and these must be available when they are needed. Rearing programs often have problems when rearing methods require unique, little-used materials since the commercial availability of these items is likely to be sporadic. Alternatives should be identified for use in the event of unforeseen shortages. Inventories

of materials such as containers, lids, and dietary ingredients are best expressed in units per 1,000 (or 1 million) insects so that potential production can be easily determined from inventory reports. When inventories reach a certain level, resupply should be automatic and in quantities required to rear a given number of insects. This way, cycles of production may begin with new inventories of supplies. Procurement and contracting processes, though time consuming, must be accounted for in a perfectly tuned inventory-control system. Otherwise, supplies will run out while resupply is being processed. A well-run inventory-control system will insure a steady supply of materials.

Automated or semiautomated insect-rearing operations that rely on costly equipment are likely to collapse when mechanical breakdowns occur. But having replacement machines is usually not possible, so parts should be stocked to facilitate onsite repairs. If a breakdown is recurrent and repair becomes routine, the machine should be replaced or modified, or a preventive-maintenance plan should be devised. Preventive maintenance is an excellent way to lengthen equipment life and avert breakdowns.

Funds.—Fiscal management means getting the greatest number of quality insects for the least money. The production manager must find low-cost supplies that conform to established specifications. These should fulfill quality requirements of the reared insects. For example, bacteriological-grade agar need not be used if a less refined grade is adequate for normal growth, development, and reproduction of the colony; or less costly gelling agents such as carrageenins may be acceptable. If not enough is known about the relationship between cost and quality, favor quality.

Careful examination of a production system often reveals many opportunities for economizing, but savings that cause the user program to fail are meaningless. In many cases, mechanization will be cost effective; time-and-motion analyses, for instance, may help identify costly operations requiring additional research to increase efficiency. At other times, simple reorganization of operating sequences or rearrangement of work areas may increase productivity.

Time, space, personnel, materials, and funds are the main resources a manager has to work with in the production program. When they are available in appropriate proportions, the rearing system operates effectively; imbalances lead to inefficiency. Therefore, each rearing program must reach its own balance and be prepared for necessary readjustments.

Operating plans

Operating plans are written prescriptions with enough detail to guide inexperienced workers through their jobs. Such a plan is commonly called a standard operating procedure (SOP); its purpose is to define and describe all jobs conducted in a systematic production program. Constructing an operating plan is difficult and requires the production manager to decide how and when certain jobs are performed. The manager should keep the SOP up to date and must insure that its guidelines are followed. The operating plan should be under constant review and should be modified as refinements are made in the rearing operations. New methods should not be introduced into the system until they have been thoroughly tested and made workable. A useful SOP has all rearing activities arranged in chronological sequence in a looseleaf folder, so modifications, additions, or deletions can be made easily.

The manager should have contingency plans prepared for use in the event the operating plan is disabled. Normally, the contingency plan will be less than ideal for several reasons, but it will help maintain production while the system is being repaired. For example, a contingency plan would allow the use of a locally available gelling agent after the stock of specified agar is depleted and before a new supply is delivered. Shortages such as these may be difficult to avoid for several reasons, and proven, available alternatives should be identified. A contingency plan should be used only when absolutely necessary.

An SOP also gives managers a written standard of employee performance, and workers have a clear understanding of what they are supposed to do. So the SOP provides managers and workers with a common ground that helps eliminate daily variations in the way certain jobs are done and helps identify areas where training can be provided to improve skills. If the manager does not use a detailed operating plan, workers will develop their own ways of accomplishing their tasks, and those methods will vary from worker to worker. Operating plans should embrace all aspects of the production program from the seemingly trivial, routine operations to those of great complexity or sensitivity.

Review

If careful attention has been given to organizing objectives, resources, and operating plans, the rearing program will probably be successful. The objectives, resource-management scheme, and operating and contingency plans are collated into a standard production work plan. Of course, the rearing operation has not been set into perpetual motion. Continual managerial guidance is required to insure that everything is going according to plan, since unforeseen and potentially catastrophic situa-

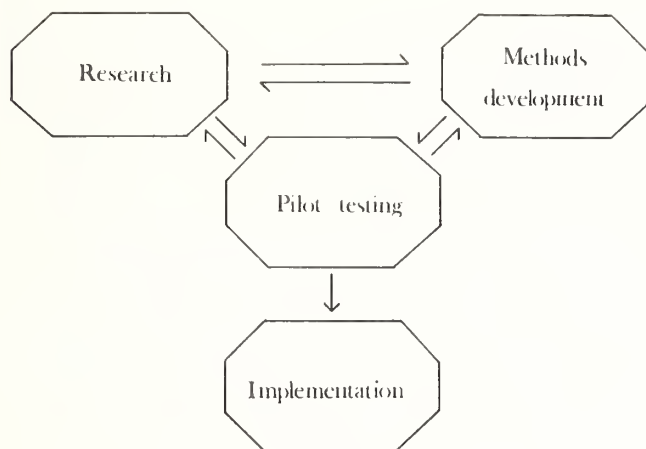


Figure 2.—Technology development and implementation.

tions can arise quickly. Most problems will be easily identifiable; solving them improves the system and prevents similar difficulties in the future. So the manager should make sure that quality is monitored and that the system is under continual review.

Intensive monitoring of insect quality is a production manager's primary means for detecting system breakdown. Other means are available; employee suggestions, safety records, and cost analyses can all be valuable indicators of various deficiencies. The manager must believe that an attempt should be made to prevent the recurrence of any complaint or technical problem no matter how small. Problems may be dealt with directly through changes in scheduling or production sequence; or they should be referred to the technology group if they are technical. Later development in research or methods may be needed; but, in many cases, data may already be available to base alternatives on. If new technology is required, it should be developed and implemented according to well-defined guidelines.

Technology development and implementation

Reports of declining yield or quality may indicate a need for improvements in operations. Operational adjustments may also be made in response to increasing costs, declining budgets, decreasing personnel ceilings, enlarged user needs, or unavailability of materials. Production managers faced with any or all of these problems use the review process to identify critical problem areas and establish their order of importance so that efforts to improve technology can be properly focused.

Technology development and its implementation in the rearing system is an interdependent process (fig. 2) with research, methods development, and pilot testing interconnected by a two-way flow of technology and information. If the process works properly, research leads to methods development, which may discover problems that require more research; if not, the new technology is pilot-tested; any problems discovered there are returned to research or methods. Finally, when all the problems are solved, the technology is implemented. Theoretically, the flow between pilot testing and implementation is one-way.

Research and development should be directed to areas where needs are greatest. The technology group must be sensitive to practical problems of production because newly produced technology is seldom incorporated into the SOP without large-scale testing and further adjustment. The manager must consider insect yield and the quality and efficiency of associated rearing operations when determining whether or not the new technology will be applicable. Only in unusual cases of need should the new technology be implemented without intermediate pilot testing.

Conclusions

If the individual overseeing the operations of insect production does not appreciate the broad scope of the assignment, day-to-day problem-solving activities will replace the more profitable long-term planning and management of the facility. Appreciating the interactions of the components as discussed in this paper will facilitate smooth operations and effective communication among all participants.

Politics in Insect Rearing and Control

The Medfly Program in Guatemala and Southern Mexico

By Patrick Patton¹

Politics play an important part in any program for insect rearing and control. Even a localized program will have internal and local politics to contend with. The larger the territory covered by a program, the more important politics become. In an international program, the scope and depth of political problems can be enormous, and they must be dealt with in many ways. The international program to control the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in Guatemala and southern Mexico is an excellent example. It is the largest program of its kind ever attempted, and political problems and their solutions have been important to the program since its inception.

The current program for controlling the Mediterranean fruit fly (medfly) is an outgrowth of political events that took place after the medfly became established in Costa Rica (fig. 1) about 1955 and soon thereafter spread to Nicaragua and Panama. Fearing that it would spread farther northward, the United States and Mexico joined with several international organizations in the mid-1960's to begin a program to eradicate the medfly from Nicaragua and maintain a barrier zone south of its border with Costa Rica. The program was successful. But, as so often happens with such delicately balanced programs, it collapsed when much of the funding was discontinued, in this case by the United States and Mexico. The medfly was still far from their borders and therefore not considered a critical problem.

Political and sociological changes in the region exacerbated the medfly problem during the next 10 years. An apparently effective quarantine between Nicaragua and Honduras began to fail as transportation of agricultural products between the countries increased. The chaos caused by the earthquake that devastated Managua, Nicaragua, in 1973 eliminated the remaining semblance of a quarantine, and the medfly invaded El Salvador and Guatemala in less than a year. The United States and Mexico again became concerned, wanting to stop the medfly before it crossed the Mexican border.

Cooperation was needed from the Governments of El Salvador and Guatemala to construct a production facility,

produce 100 million sterile flies per week, and create another barrier. But, in 1975, the Government of El Salvador was unstable, the country on the brink of revolution. Guatemala was an alternate possibility, but its main crops were not heavily affected by the medfly and were by other pests, so it was not very interested in the problem. Guatemala did, however, want Mexican oil, and this became a bargaining chip. So, in 1977, Guatemala and Mexico formed the Comisión Moscamed to combat medflies in Guatemala.

The political negotiations that established this commission were concluded as the first medfly was discovered along the northern side of the Rio Suchiate between Mexico and Guatemala. By the end of 1977, 122 flies had been detected. In the face of this invasion, Mexico sought financial and technical support for a program to drive the medfly out. Several national and international organizations were approached. These included the Organization of American States, the International Atomic Energy Agency, the Food and Agriculture Organization (FAO), and the agriculture and state departments of concerned countries. After much negotiation, the program was financed by means of a cooperative agreement that provided for Mexico and the United States each to cover half the cost. Several of the international organizations were also to participate in the program. It was decided to construct a production facility at Metapa, Mexico; establish a barrier zone between Mexico and Guatemala; and eradicate the medfly from north of this border. The facility began production in March 1979 and was producing 500 million sterile flies per week by 1980.

One of the political problems in an international program is that opinions about how things should be done must be handled with diplomatic sensitivity. When conflicting advice comes from many nations and regions whose participation is necessary, someone must resolve the conflicts tactfully and without harming the program. In the medfly program, this problem is intensified because the facility is managed by a group of people whose average age is under 30, a perceived handicap that means they have to defend their actions constantly and spend extra time maintaining their credibility. The challenge for this or any group is to evaluate available information and adopt or modify the most appropriate technology without making any enemies by rejecting someone's advice. So, in the medfly program, the Mexican general coordinator reviews all suggestions and plans coming from various

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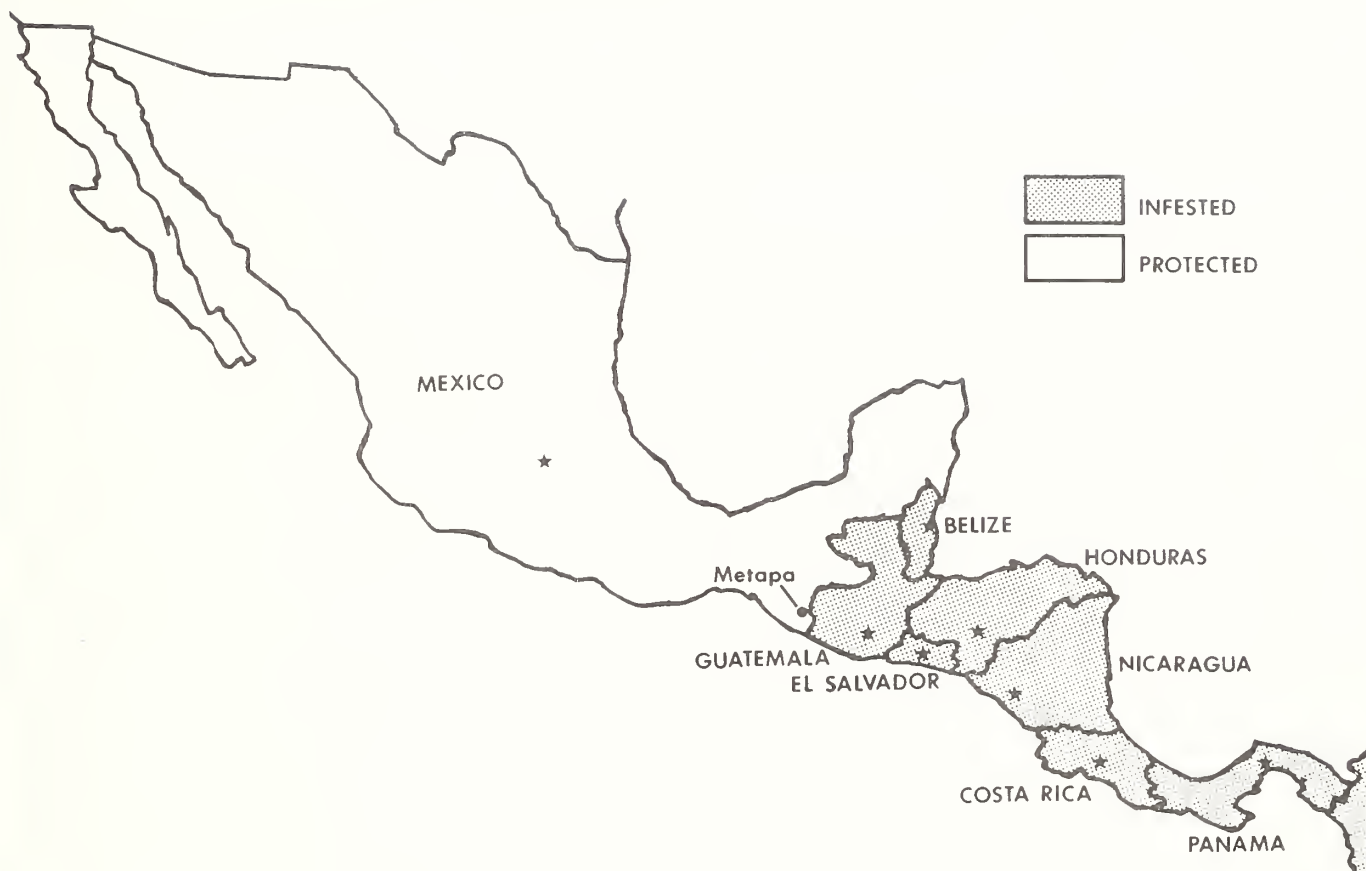


Figure 1.—Areas of Central America and Mexico infested by and protected from the medfly.

people and agencies and then makes the decisions in conjunction with his counterparts. Dealing with the political problems in this way, the Metapa group adapted the rival Hawaiian and Viennese mass-rearing systems to the much larger production quotas of Metapa and emerged with the Metapan system.

These problems stem from the international aspects of the medfly program. But, as with all insect-rearing programs, local politics are also important. A good way to avoid local political problems is to keep everyone affected by a program informed about its purposes and methods. But doing so is often difficult for the medfly program because it encompasses diverse geographical areas, climates, human cultures, agricultural practices, and fly habitats. For example, consider a massive bait-spray and sterile-insect release operation conducted along the border between Mexico and Guatemala. The spray was 800 ml of

protein hydrolyzate plus 200 ml of malathion² per hectare. The people living in the area did not understand that this spray was not dangerous to them, their crops, or their animals; nor did they understand the importance of bait spraying to medfly control. So, whenever an airplane was spraying, they would hide. And they blamed the spray for everything from dead chickens to the common cold. They were also afraid that this was some drastic governmental form of birth control. Politicians played on this fear to win votes; and newspapers, for their own reasons, printed many negative articles about the whole program. At one point, concerned about the failure of their drought-stricken crops, 4,000 farmers stormed my office at the production facility, blaming

²O,O-Dimethyl phosphorodithioate of diethyl mercaptosuccinate.

their problems on the bait spraying. So, even though efforts had been made from the beginning to educate the people, a more intensive information campaign was developed to explain the benefits of controlling the medfly.

The political problems inherent in such a program will always be there. Program managers must, therefore, be politicians. They have to convince potential funding

sources that the program is needed and can be successful. They have to coordinate the contributions of program participants with the skill of seasoned diplomats. They have to insure that the public is well-informed about what the program is doing and why. In short, there is more to managing an insect-rearing program than just rearing insects.

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